

Dissertation

Development of an HPLC-fluorescence method for polycyclic aromatic hydrocarbons and their nitro-derivatives in atmospheric particulate matter and its application

大気粒子中の多環芳香族炭化水素及びそのニトロ誘導体の測定のための蛍光 HPLC 法の開発とその応用

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Dissertation Summary

My dissertation focuses on development of an analytical method for polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs) in atmospheric particulate matter (PM). Analytical methods for PAHs and NPAHs in the PM samples have been widely studied because of their carcinogenicity and mutagenicity. PAHs and NPAHs are mostly determined by gas chromatography (GC) and high-performance liquid chromatography (HPLC). In the reported GC methods using mass spectrometry (MS), PAHs and NPAHs are detected with electron impact ionization (EI) and negative ion chemical ionization (NICI) modes, respectively. GC-MS methods have high sensitivity and selectivity, but have limitations in the separation of several PAHs and difficulty in detecting PAHs with NICI mode. On the other hand, HPLC methods with chemiluminescence detection (HPLC-CL) have been successfully used for NPAH analysis. However, the CL system is complicated by the extra equipment needed for post-column CL reagents, which consume an especially large amount of acetonitrile. Furthermore, the CL detection cannot be applied to PAH analysis because their fluorescence characteristics are inadequate for the CL detection. To overcome the disadvantages of the CL methods, HPLC methods with fluorescence detection (HPLC-FL) are preferable for the analysis of PAHs and NPAHs. The facts motivated me to develop an HPLC-FL method for simultaneous determination of PAHs and NPAHs in atmospheric PM.

My dissertation consists of two steps: (1) Development of an HPLC-FL method for PAHs and NPAHs; (2) Application of the developed method to atmospheric PM samples. The first step was the development of an HPLC-FL method for simultaneously analyzing 10 PAHs and 18 NPAHs. The two-dimensional HPLC system consists of an on-line clean-up and reduction for NPAHs in the 1st dimension, and separation of the PAHs and the reduced NPAHs and their FL detection in the 2nd dimension after column-switching. To identify an ideal clean-up column for removing sample matrix that may interfere with detection of the analytes, the characteristics of 8 reversed-phase columns were evaluated. The nitrophenylethyl (NPE)-bonded silica column was selected because of its shorter elution band and larger retention factors of the analytes due to strong dipole-dipole interactions. After column switching, the amino-substituted PAHs (reduced NPAHs), PAHs and deuterated internal standards were successfully separated on polymeric octadecyl-bonded silica (ODS) columns and by dual-channel detection within 120 min including the 1st dimension. The analytical time for NPAHs was reduced by half compared to the HPLC-CL method and was followed by PAH analysis. The two-channel program was set based on optimal excitation and emission wavelengths of the analytes. Remarkably, 6-nitrochrysene and 7-nitrobenz[*a*]anthracene were separated by their specific excitation and emission wavelengths, not by the separation columns as they had identical retention times. The signal arising from one analyte was not detected on the other channel. These results suggest that the proposed HPLC-FL system is suitable for the identification of all target compounds. The limits of detection were 0.1-9.2 pg per injection for PAHs and 0.1-140 pg per injection for NPAHs. The second step was the application of the developed method to crude extracts of fine particulate matter (PM_{2.5}) samples and the standard reference material of urban dust (SRM1649b). The extraction procedure of PAHs and NPAHs in PM samples involved ultrasonic extraction with dichloromethane, evaporation and redissolving steps, and then the crude extract was directly injected to the HPLC-FL system. The analytical precision and accuracy were satisfactory for the simultaneous determination of all PAHs and NPAHs in the PM_{2.5} samples and the observed concentrations of them in SRM1649b samples were similar to those in previous reports. Thus, the method developed herein has the potential to become a standard HPLC-based method, especially for NPAHs.

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List of abbreviation

Ant	Anthracene
BaA	Benz[<i>a</i>]anthracene
BaP	Benzo[<i>a</i>]pyrene
BbF	Benzo[<i>b</i>]fluoranthene
BghiPe	Benzo[<i>ghi</i>]perylene
BkF	Benzo[<i>k</i>]fluoranthene
Chr	Chrysene
CL	Chemiluminescence
CO ₂	Carbon dioxide
DBA	Dibenz[<i>a,h</i>]anthracene
DCM	Dichloromethane
DHHS	The Department of Health and Human Services
DMSO	Dimethyl sulfoxide
DNP	Dinitropyrene
EPA	Environmental Protection Agency
FL	Fluorescence
Flu	Fluoranthene
GC	Gas Chromatography
GC-MS	Gas Chromatography-mass spectrometry
GC-EI-MS	Gas Chromatography-mass spectrometry with electron ionization
GC-NICI-MS	Gas Chromatography-mass spectrometry with negative-ion chemical ionization
HPLC	High Performance Liquid Chromatography
IARC	The International Agency for Research on Cancer
IDP	Indeno[1,2,3- <i>cd</i>]pyrene
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass Spectrometry
NAAQS	National Ambient Air Quality Standards
NBaA	Nitrobenz[<i>a</i>]anthracene

NBaP	Nitrobenzo[<i>a</i>]pyrene
NC	Nitrochrysene
NFR	Nitrofluoranthene
NP	Nitropyrene
NPAHs	Nitropolycyclic aromatic hydrocarbon
NPhe	Nitrophenanthrene
NIST	National Institute of Standards and Technology
PAH	Polycyclic aromatic hydrocarbon
PM	Particulate matter
PM _{2.5}	Fine particulate matter (Particulate matter less than 2.5 micrometers)
Pyr	Pyrene
SD	Standard deviation
SRM	Standard reference material
TSP	Total suspended particulate matter
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

Chapter 1

Introduction

1.1 Background information

Air pollution become serious issue because exposure of air pollution caused health problem and among policy maker and many researchers are an increasing awareness about their effective. Air pollution contaminated into the indoor or outdoor environment by any chemical, physical or biological agents that modifies the natural characteristics of the atmosphere, residence, vehicles, industrial process and forest fires are common sources of air pollution. Pollutants of major public health concern include particulate matter, carbon monoxide, ozone, nitrogen dioxide sulfur dioxide and lead. Air pollution cause respiratory and other diseases, which can have a fatal impact on human health. The US EPA or United States Environmental Protection Agency has established air quality standard for six common air pollutants to protect public health and environment. These substances have been used as indicators of air quality. High contamination of the air pollutants (poor air quality) can cause health problem to human. For example, its aggravate existing respiratory diseases such as asthma and bronchitis, or increase the risk of respiratory problems. Additionally, air quality is important to protect and prevent the life environment from harmful and unhealthy levels of air pollution, the governments of various countries have adopted policy and regulation to protect the health and wellbeing of humans, ecosystem and animals. Air quality standards have been set as limits on emission and air pollution in various areas in the world.

Particulate matter or PM contains microscopic solids or liquid droplets on the order of micrometers that can be inhaled and cause serious health problems and air pollutants. PM can be classified by the particles size and it is directly linked to their potential for causing health problems. PM_{2.5} or fine particles less than 2.5 μm (PM_{2.5}) in diameter pose the greatest health problems because they can get deep into lung, and some may even get into your bloodstream. A long-term exposure to fine particles leads to more serious chronic disorders than ground-level ozone and other air pollutants such as carbon monoxide (CO). PM is a heterogeneous mixture of solid and liquid particles suspended in air, which varies continuously in chemical composition and size fraction depending on time and space. It found that the chemical constituents of PM are diverse and they consist nitrate, sulfates, elemental carbon, organic carbons such as organic compounds (e.g. polycyclic aromatic hydrocarbons (PAHs)) and biological compounds (e.g. endotoxin and cell fragments) and metals (e.g. iron, copper, nickel, zinc, and vanadium). Many researchers have studied and tried to explain PM adverse effects including oxidative damage, enhancement of the inflammatory response, cytotoxicity and mutagenicity that ultimately lead to chronic diseases, increased morbidity, and mortality. The mechanisms involved in these toxic effects and the contribution of constituents of PM such as PAHs to overall toxicity of PM is still poorly understood. Moreover, PM have been well-studied for source distribution, formation, fate, behavior, size fractionation, analytical method and environmental monitoring and it is well known that the status of PM pollution is highly significant in some Asian countries.

1.2 Atmospheric particulate matter (PM)

Atmospheric particulate matter (PM) can be emitted by a large variety of sources which influence its chemical composition, physical properties (size, surface area and density) and size distribution. In general, Atmospheric PM sources are classified by two main sources as primary or secondary formations in accordance with its formation mechanism. PM directly emitted into the atmosphere from primary natural and anthropogenic sources. The most noteworthy anthropogenic sources are incomplete combustion processes of fossil fuels and biomass (Grudzinski, 2007). On the other hand, secondary sources of particles are formed after chemical transformation of their gaseous precursors (Perrino, 2010).

Atmospheric PM is classified by particle size (aerodynamic diameter). Several metrics for PM have been used. PM_{10} and $PM_{2.5}$ is PM with an aerodynamic diameter of less than 10 and 2.5 μm , which have serious problems because easily to get deep into human lung, respectively. The $PM_{2.5}$ fraction is also called “fine particles”, and particles between 2.5 and 10 μm are currently named “coarse particles”. Air Quality Standards have been set in the world such as the United States, European union (EU), China, Hong Kong, Japan, South Korean and Australia for considered to be a basic requirement of human health, environment. The summary of regulations for PM in the world were listed in Table 1.1 (Kim et al., 2015). Numerous researchers in Asia, US and Europe have reported effects of PM level to human health. PM are related to respiratory symptoms, chronic bronchitis, cardiovascular diseases, premature mortality (Kappos et al., 2004; Englert et al., 2004; Samoli et al., 2008; Polichetti et al., 2009; Cheng et al., 2013).

Table 1.1 Atmospheric particulate matter (PM) regulation guideline set by various governments

Order	Country	Period	PM_{10} ($\mu g m^{-3}$)	$PM_{2.5}$ ($\mu g m^{-3}$)
1	United	Yearly average	None	12
		Daily average (24-hour)	150	35
2	European Union	Yearly average	40	25
		Daily average (24-hour)	50	None
3	China	Yearly average	70	35
		Daily average (24-hour)	150	75
4	Hong Kong	Yearly average	50	35
		Daily average (24-hour)	100	75
5	Japan	Yearly average	None	15
		Daily average (24-hour)	100	35
6	South Korea	Yearly average	50	25
		Daily average (24-hour)	100	25
7	Austrial	Yearly average	None	8
		Daily average (24-hour)	50	25

1.3 Toxicity of PAHs and NPAHs

One of the typical hazardous organic compounds in PM is polycyclic aromatic hydrocarbon (PAHs) and their nitro-derivatives as nitropolycyclic aromatic hydrocarbon (NPAHs). They are of great concern due to their carcinogenic and/or mutagenic activities to human (Ames et al., 1975). In general, atmospheric PAHs and NPAHs are primarily generated from the incomplete combustion of organic matter. Several NPAHs are appeared by the reaction of parent PAHs with nitrate radicals or NO_3 in the nighttime and the reaction with hydroxyl radicals or OH in the presence of NO_x in daytime (Wu et al., 2012). PAHs and NPAHs having strong carcinogenic and/or mutagenic activities are mainly associated with PM and especially some NPAHs have specific concern because the shown strongest direct-acting carcinogenicity and/or mutagenicity compared to their parent PAHs (Hayakawa et al., 1995).

Polycyclic aromatic hydrocarbon (PAHs) are a large group of hazardous organic compounds with at least two or more fused aromatic (benzene) rings. Low molecular weight (2 and 3 rings) of PAHs in the atmosphere are predominantly existed in vapor phase, whereas high molecular weight (5 ring or more) of PAHs are largely bound to atmospheric PM. Intermediate molecular weight (4 rings) of PAHs are partitioned between the vapor and particulate phases, depending on the atmospheric temperature. PM-bound PAHs has considered to be serious hazardous to human health (Choi et al. 2010). Typical PAHs observed in atmosphere are shown their structure and abbreviations in Fig. 1.1. Moreover, the Department of Health and Human Services (DHHS) has determined that benz[a]anthracene (BaA), benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene are identified as animal carcinogens. The International Agency for Research on Cancer (IARC) has determined as follows; BaP (Group 1, carcinogenic to human); DBA (Group 2A, probably carcinogenic to humans); BbF, BkF, IDP (Group 2B, possibly carcinogenic to humans). EPA has determined that BaA, BaP, benzo[b]fluoranthene, BkF, Chr, DBA, and IDP are probable human carcinogens and that Ace, Ant, BgPe, Fle, Flu, phe, and Pyr are not classifiable as to human carcinogenicity. The DHHS, IARC, or EPA are identified Ace has not been classified for carcinogenic effects (Mumtaz et al. 1995). The carcinogenic classification of 16 priority PAHs by the IARC, the US EPA and the DHHS shows in Table 1.2.

Additionally, In European found BaP is associated with fine particulate matter and has the highest carcinogenic potency. It has been recognized as the most toxic PAHs and standard values have been defined in several countries to protect human health. The European Commission has fixed a target value of 1 ng m^{-3} for the annual average concentration of BaP in ambient air (European Environment Agency, 2015). The concentrations of PAHs in ambient air including BaP are of increasing concern in Europe due to the BaP levels have been relatively higher concentrations than the target values. During the year of 2011-2013, the EU was found to be exposed to BaP about 25–29% of the urban population over the above mention target values. Considering the WHO reference level of 0.12 ng m^{-3} , 85-91% of the urban populations in the EU are being exposed to BaP at the concentration higher than the reference level. This reference level was estimated by assuming the WHO unit risk for lung cancer through exposure to PAH mixtures and an acceptable risk of additional lifetime risk of approximately 1×10^{-6} (Hellen et al., 2017). Zhu et al., (2011) measured BaP concentrations and found that the average ambient concentration of BaP in an urban community and Village of Waterfront South (WFS) are contaminated with various sources of air toxicity in

Camden, New Jersey (0.36 ng/m^3) were higher concentrations than the standard value (0.25 ng/m^3) in United state.

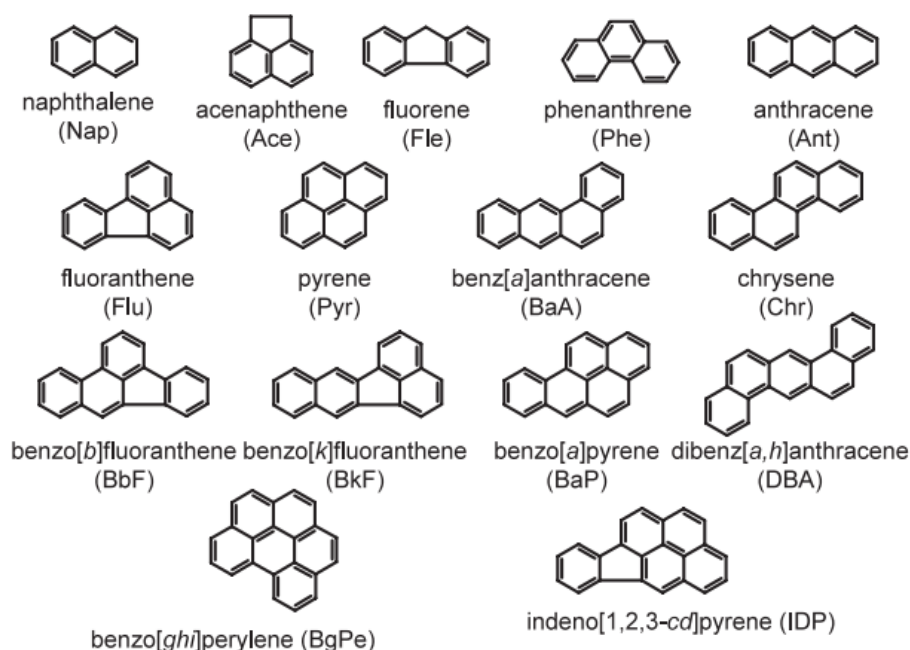


Fig. 1.1 Structures and abbreviations of PAHs classified as priority pollutants by USEPA (Hayakawa, 2009)

Table 1.2 Carcinogenic classification of 16 priority PAHs classified by IARC, US EPA and DHHS.

PAHs	No. of ring	US EPA	IARC	DHSS
Nap	2		2B	
Acl	3	Not classifiable		
Ace	3		3	
Fle	3	Not classifiable	3	
Phe	3	Not classifiable	3	
Ant	3	Not classifiable	3	
Flu	4	Not classifiable	3	
Pyr	4	Not classifiable	3	
Chr	4	Probably carcinogen	2B	
BaA	4	Probably carcinogen	2B	Animal carcinogen
BbF	5	Probably carcinogen	2B	Animal carcinogen
BkF	5	Probably carcinogen	2B	
BaP	5	Probably carcinogen	1	Animal carcinogen
DBA	5	Probably carcinogen	2A	Animal carcinogen
BgPe	6	Not classifiable	3	
IDP	6	Probably carcinogen	2B	Animal carcinogen

IARC classification; Group 1 (carcinogenic), 2A (probably carcinogenic), 2B (possible carcinogenic), 3 (not classifiable)

Recently, NPAHs can be found in trace amounts in most of environmental compartments. In recent years, increasing attention has been concerned on NPAHs because of their higher mutagenicity (2×10^5 times) or carcinogenicity (10 times) than their parent-PAHs. (Zhang et., al 2014). NPAHs are derivative of PAHs and they are a class of aromatic compounds with containing at least one nitro-functional group on the aromatic ring and Fig. 1.2. shown structures and abbreviations of typical NPAHs observed in atmosphere. NPAHs can either be directly emitted from combustion sources such as diesel and gasoline engines and formed in the atmosphere via their parent PAHs reactions with OH or NO₃ radical. (Pitts, 1977). Meanwhile, the IARC has classified 1,3- 1,6- and 1,8-dinitropyrene (1,3-,16- and 1,8-DNPs) as Group 2B and 1-nitropyrene (1-NP) as Group 2A (IARC, 2013). Carcinogenic classification of typical NPAHs by IARC shows in table 1.3. The widely accepted short-term assay, Ames test is an indicator for DNA damage leading to gene mutation which could be linked to cancer. Parent PAHs are indirect-acting mutagens, requiring metabolic activation to convert them into active forms (Jariyasopit, 2013). Most NPAHs are direct-acting mutagens which are independent of metabolic activation, with an exception of 6-nitrobenz[*a*]pyrene and 1-nitrocoronene (IPC 2003). Some NPAHs were found to be more toxic than their parent PAHs. For example, DNPs were found to be very powerful direct-acting mutagens, greatly exceeding that of pyrene (Hayakawa et al., 1995; Jariyasopit, 2013). Therefore, extremely low concentrations of some NPAHs in the environment, compared to PAHs, would show a large contribution to total mutagenicity. Although the mutagenicity of DNPs were greater than that of BaP, the lack of their carcinogenicity studies on humans resulted in DNPs being classified as Group 2B, “possibly carcinogenic to humans” by IARC.

Table 1.3 Carcinogenic classification of typical NPAHs by IARC

NPAHs	No. of ring	Carcinogenic group classification
1,6-DNP	4	2B
1,8-DNP	4	2B
1,3-DNP	4	2B
2-NF	3	Not classifiable
9-NA	3	Not classifiable
9-NPh	3	Not classifiable
2-NA	3	Not classifiable
1-NFR	4	Not classifiable
2-NFR	4	Not classifiable
3-NFR	4	Not classifiable
4-NP	4	2B
1-NP	4	2A
2-NP	4	3
7-NBaA	4	3
6-NC	4	2A
1-NPer	5	Not classifiable
6-NBaP	5	3
3-NPer	5	3

IARC classification; Group 1 (carcinogenic to human), 2A (probably carcinogenic to humans), 2B (possible carcinogenic to humans), 3 (not classifiable as to its carcinogenicity to humans), 4 (probably not carcinogenic to humans)

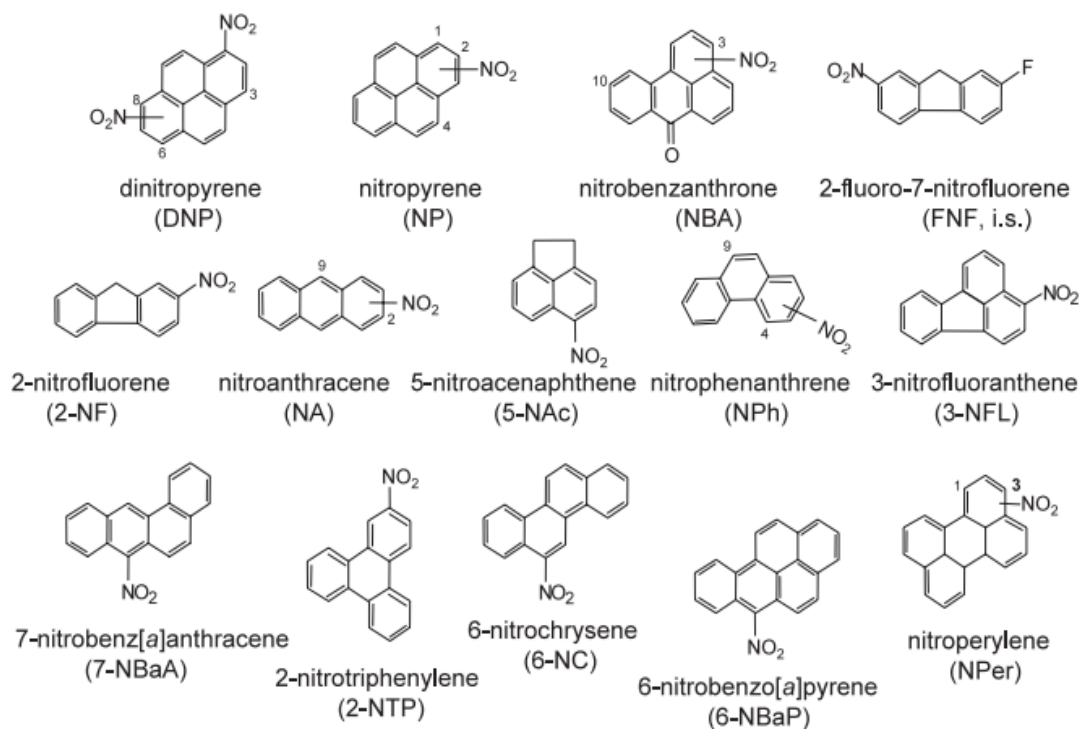


Fig. 1.2 Structures and abbreviations of typical NPAHs observed in atmospheric (Hayakawa, 2009)

1.4 Source, formation and behavior of PAHs

PAHs are emitted to the atmosphere primarily from the incomplete combustion of organic materials. The combustion sources can be either natural or anthropogenic. The natural sources are such as from volcanoes and forest fires, whereas the anthropogenic sources are vehicle exhaust, agricultural fires, power plants, coke plants, steel plants, foundries and industrial sources (Golomb et al., 2001; Halsall et al., 2001; Garban et al., 2002; Shafy et al., 2015). PAHs are ubiquitously distributed in atmosphere due to their widespread sources and behavior such as long-range transportation. PAHs consist of carbon and hydrogen atoms grouped and are a large group of chemicals at least 2 to 7 fused aromatic rings (Kim et al., 2013). However, PAHs can be decomposed through photochemical reactions in the atmosphere under strong ultraviolet light and/or sunlight. Additionally, PAHs can react with hydroxyl radicals, ozone, sulfur oxide, nitrogen, nitric and sulfuric acids which affect the environmental fate of PAHs (Lee and Vu, 2010).

The physicochemical properties of the PAHs have a large effect on their environmental behavior. Some PAHs are semivolatile substances and frequently existed both in the vapor and particle phases, depending on the vapor pressure of each PAH and atmospheric conditions (Wingfors et al., 2001; Basheer et al., 2003). PAHs can be divided into two categories which are low molecular weight PAHs (LMW) and the high molecular weight of PAHs (HMW). PAHs are hydrophobic and lipophilic compounds which increase as the number of rings increases. Physicochemical properties of 16 PAHs are shown in Table 1.4. The PAHs has been classified as priority pollutants based on their toxicity by the US EPA (US EPA 1982).

PAHs tend to be observed in higher concentrations in urban environments than those in rural environments because most PAHs sources are located in the local area. PAH concentrations in the gas phase increase in summer or in tropical regions, whereas particulate phase PAHs are dominant

during in winter or in cold region throughout the year (Lai et al., 2011; Mohanraj et al., 2012). Furthermore, when industrial emissions are a major source of PAHs, their concentrations show little seasonal variations because the emission is constant throughout the year (Kim et al. 2013). However, in most urban, residential and rural areas where the local sources are related to residential and commercial heating, they show significant seasonality during the year (e.g. relative enhancement during the cold winter). This effect is particularly pronounced when the fuels used for heating indicating high PAH emission factors. A clear example was found in Northern Ireland, UK where solid fuels such as coal and wood are still commonly burnt for heating (Lisowska et al. 2005). The average BaP concentration in December to January and June to August were 3.0 ng/m^3 and 0.19 ng/m^3 , respectively. Moreover, PAH adsorption onto particle phase depends on humidity (Zhang et al., 2009) and the type of suspended particulates such as soot, dust, fly-ash, pyrogenic metal oxide pollens, etc (Li et al., 2011).

The background level of some representative PAHs was reported values to be 0.02 to 1.2 ng/m^3 in rural areas and 0.15 to 19.3 ng/m^3 in urban area (Huertero et al., 2011), and urban background in 120 US cities fluctuated between 0.15 - 19.3 ng/m^3 (ATSDR, 1995). Legzdins et al. (1994) was studied about PAHs and determined that BgPe was the highest individual PAH concentrations among PAHs measured in Hamilton, Ontario and New York City. The specific values, 4.3 ng/m^3 in Hamilton and 4.05 ng/m^3 in New York City, supported the notion that PAH levels of urban ambient air show a general pattern when free of point source contributions. A worldwide study of 60 towns in the mid-1970's determined BaP concentrations in US and European ambient air. The levels in the US and Europe are approximately 1 ng/m^3 and a range of 1 - 20 ng/m^3 , respectively. (Menichini, 1992). However, the range of BaP in Europe was significantly improved in the 1990's (EU, 2001). Rural background levels varied between 0.1 and 1 ng/m^3 , and urban baselines ranged from 0.5 to 3 ng/m^3 . Nonetheless, the EU (2001) reported 30 ng/m^3 as the commonly accepted background concentration around industrial areas.

Table 1.4 Physical-chemical characteristics of some PAHs

PAHs	M.W. (g/mol)	Solubility (mg/L)	Vapor pressure P (Pa)	Log K_{ow}
Nap	128	32.00	11	3.4
Acl	152	3.90	9.0×10^{-1}	4.1
Ace	154	3.90	3.0×10^{-1}	3.9
Fle	166	1.90	9.0×10^{-2}	4.2
Phe	178	1.10	2.0×10^{-2}	4.5
Ant	178	0.05	1.0×10^{-3}	4.5
Flu	202	0.26	1.23×10^{-3}	5.2
Pyr	202	0.13	6.0×10^{-4}	5.2
Chr	228	0.002	1.4×10^{-6}	5.9
BaA	228	0.01	2.8×10^{-5}	5.6
BbF	252	0.0014	6.7×10^{-5}	5.8
BkF	252	0.0007-0.008	5.2×10^{-8}	6.0
BaP	252	0.003	7.0×10^{-7}	6.0
DBA	278	0.0005	3.7×10^{-8}	6.5
BgPe	278	0.000260	1.4×10^{-9}	7.1
IDP	276	0.000190	1.3×10^{-8}	6.6

1.5 Source, formation and behavior of NPAHs

NPAHs are derivatives of PAHs containing at least one nitro-functional group on the aromatic benzene ring of a PAH (Bandowe et al., 2017). As mentioned above, NPAHs are mainly generated by incomplete combustions and also formed by atmospheric reactions of gas- and particulate-phase PAHs with oxidants, (Schuetzle et al., 1989; Araki et al., 2009). The physico-chemical properties of NPAHs which commonly found in the environment are affected by the molecular weight (MW) and number of nitro-groups (Table 1.5). The physico-chemical properties of NPAHs listed on Table 1.5 were determined by different experimental methods, and modeling approaches, which also lead to inconsistent trends and makes the datasets not completely comparable (Yaffe et al., 2001; IPCS, 2003). With increasing MW, NPAHs have higher melting points (Mp), octanol-water partition coefficients (K_{OW}) octanol-air partition coefficients (K_{OA}), organic carbon-water partition coefficient (K_{OC}) and particle-gas partition coefficients (KP), but lower vapor pressures (Vp), water solubility (S) and Henry constants (H). These properties drive the fate, transport, partition, and transfer of NPAHs within and between environmental compartments (gas phase, particle phase, water-dissolved phase, water suspended particles, sediments, biota and soil) and their (eco)toxicological effects. NPAHs have higher MW, KOA, KP, and lower Vp, S, KOW, KOC and H than their related PAHs. Generally, a nitro-PAH derived from single NO_2 -functional group substitution on a PAH reduces the Vp and S by 3 and 1 order of magnitude, respectively (Yaffe et al., 2001). The KOW can generally decrease (by 25–80%) with nitro-group substitution on PAHs (IPCS, 2003; Yaffe et al., 2001). The presence of nitro-group leads to a decrease in S compared to their related PAHs as shown in Table 1.5, which can be explained by the higher MW of nitro-PAHs. The decreases in KOW, KOC and H (compared to related PAHs) are consistent with the presence of the more polar nitro-groups. However, the higher KOA of nitro-PAHs compared to their related PAHs, indicates that the introduction of nitro-groups leads to higher partition into a more hydrophobic octanol phase. The effects of the presence of nitro-groups on a PAH on the physical and partition properties is complicated, driven by several factors not necessarily related to the known relationships between polarity of molecules, aqueous solubility and partition into hydrophobic media like octanol (Bandow et al., 2017). NPAHs with 2 aromatic rings were detected in the vapor phase (gas-partition), and three rings NPAHs were found in both gas and particle phase in the atmosphere. On the other hand, NPAHs with 4 or higher ring were mainly distributed in particle phase (Dimashki et al., 2000).

NPAHs have been primarily detected in airborne PM, and their concentrations in urban areas are higher than those in suburban areas. However, their concentrations range from pg/m^3 to ng/m^3 and are significantly lower than their parent PAH concentrations. (Jariyasopit, 2008; Garcia et al., 2014). NPAHs distributed in the particulate phase are primarily dominated by the NPAHs formed through atmospheric gas-phase reactions (Jariyasopit 2008). A substantial percentage of NPAHs containing 2- 4 benzene rings in the atmosphere are formed from gas-phase reactions of parent PAHs with atmospheric oxidants (OH , O_3 , NO_x ; Dimashki et al., 2000; Atkinson and Arey, 1994; Arey et al., 1986). These radicals are unstable during daytime due to the fast photolysis of NO_3 . After sunset the concentration rises to an average of 5×10^8 molecules/ cm^3 (Atkinson and Arey, 1994). Both nitronaphthalene isomers, which are formed in similar yields, are not the main products of the OH -initiated reaction, but hydroxynaphthalene and 1,4-napthoquinone. NO_3 -initiated reactions lead to 16% of 1-nitronaphthalene and 7% of 2-nitronaphthalene (Vione et al., 2004; Atkinson et al., 1987).

The radical initiated reactions are postulated to proceed through initial addition of either the NO_3 radical or the OH radical to the position with the highest electron density in the PAH molecule (e.g. the one position from pyrene and the three positions of fluoranthene) as seen in Fig. 1.3. This followed by the addition of NO_2 at the ortho-position and subsequent loss of water or nitric acid to form 2-nitrofluoranthene (2-NFR) or 2-nitropyrene (Arey et al., 1986). 2-NFR and 2-NP have not been identified in other combustion products or diesel exhaust. Moreover, gas phase reaction in the laboratory studies have reported NPAH production via heterogeneous reaction (Pitts et al., 1985;

Kamens et al. 1990). Generally, 2-nitrofluoranthene showed the most abundant NPAH in ambient atmosphere (Zimmermann et al., 2013). NPAH isomers formed in the atmosphere can be distinguished from those formed from chemical reactions in combustion processes, in view of their formation mechanisms. For example, imperfect combustion processes that result in the formation of NPAH isomers are formed via electrophilic nitration, such as 3-NFR and 1-NP from the most abundant PAHs with four rings (Pyr and Flt) (Gracia et al., 2014). Especially, 3-NFR and 1-NP are not easily formed through gas phase reaction, and almost of them exist exclusively in particulate phase.

NPAHs has been interested studies by several researchers because of their behavior in the environment and NPAHs are shown higher carcinogenic and mutagenic properties of certain compounds compared to their parent PAHs (Bandowe et al., 2017). NPAHs also showed strong exhibit direct-acting mutagenic potency in a microbial bioassays and mutation on assay based on human cells (Jariyasopit, 2008). Significant portion of mutagenicity of the extract of atmospheric samples have attributed to NPAHs such as 1,3-, 1,6- and 1,8-DNPs, 1,3,6-trinitropyrene, 3,9-dinitrofluoranthene and 3,6-dinitrobenzo[*e*]pyrene (Hasei et al., 2006, 2009; Watanabe and Hirayama, 2001; Watanabe et al., 2005, 2008; Wang et al., 2011; Umbuzeiro et al., 2008). The toxic properties of NPAHs have been assessed and assigned toxicity equivalency factors (TEFs) much higher than their related PAHs (IARC, 1989, 2012; Collins et al., 1998).

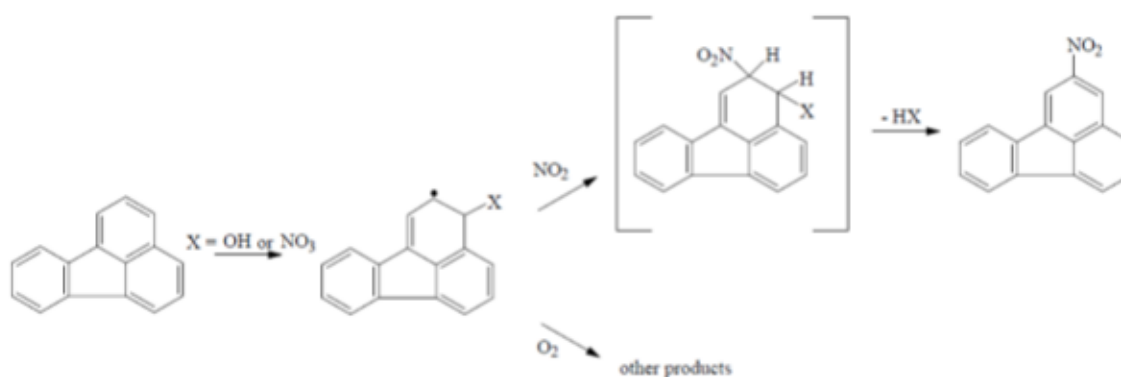


Fig. 1.3 Gas phase reaction of 2-nitrofluoranthene (2-NFR)

Table 1.5 Physical-chemical characteristics of typical NPAHs

NPAHs	M.W. (g/mol)	Melting point (°C)	Solubility (mg/L,25°C)	Vapor pressure (Pa,25°C)	Henry constant (Pa m ³ /mol)	Log Log K _{OW}	Log K _{OA}	Log K _{OC}	Kp (m ³ /μg)
1,6-DNP	292.25	204.76	5.4×10 ⁻²	1.21×10 ⁻⁷	6.57×10 ⁻⁴	4.57	12.85	3.89	1.73
1,8-DNP	292.25	204.76	5.4×10 ⁻²	1.21×10 ⁻⁷	6.57×10 ⁻⁴	4.57	12.85	3.89	1.73
1,3-DNP	292.25	204.76	5.4×10 ⁻²	1.21×10 ⁻⁷	6.57×10 ⁻⁴	4.57	12.85	3.89	1.73
2-NF	211.22	157.00	0.22	5.9×10 ⁻⁴	7.78×10 ⁻²	3.37	7.94	3.01	2.13×10 ⁻⁵
9-NA	223.23	146.00	0.115	1.60×10 ⁻⁴	4.12×10 ⁻¹	4.78	9.86	3.79	1.78×10 ⁻³
9-NPh	223.23	141.62	0.292	1.79×10 ⁻⁴	1.38×10 ⁻¹	4.16	9.24	3.44	4.28×10 ⁻⁴
2-NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
1-NFR	NA	NA	NA	NA	NA	NA	NA	NA	NA
2-NFR	NA	NA	NA	NA	NA	NA	NA	NA	NA
3-NFR	247.26	170.56	1.95×10 ⁻²	7.36×10 ⁻⁶	2.68×10 ⁻²	4.75	10.62	3.77	1.03×10 ⁻²
4-NP	247.26	170.56	6.79×10 ⁻²	5.52×10 ⁻⁸	2.68×10 ⁻²	4.75	10.93	3.77	1.03×10 ⁻²
1-NP	247.26	155.00	1.18×10 ⁻²	1.11×10 ⁻⁵	7.39×10 ⁻²	5.06	10.93	3.94	2.11×10 ⁻²
2-NP	247.26	170.56	6.79×10 ⁻²	7.36×10 ⁻⁶	2.68×10 ⁻²	4.75	10.93	3.77	1.03×10 ⁻²
7-NBaA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6-NC	273	187	1.53×10 ⁻²	1.01×10 ⁻⁶	1.82×10 ⁻²	5.34	11.43	4.1	6.64×10 ⁻²
1-NPer	NA	NA	NA	NA	NA	NA	NA	NA	NA
6-NBaP	297.32	211.87	3.49×10 ⁻³	4.14×10 ⁻⁸	3.52×10 ⁻³	5.9	12.81	4.42	1.6
3-NPer	NA	NA	NA	NA	NA	NA	NA	NA	NA

1.6 Analysis of PAHs and NPAHs in atmospheric pollutant

The determination of PAHs and NPAHs in airborne PM are carried out with similar steps. There are three main steps; (1) sample collection and extraction steps, (2) the clean-up and fractionation steps, (3) The identification and quantification of the target compounds. (Bin et al., 2007; Bandown et al., 2017). Fig 1.4 shows the three main steps for the determination of PAHs and NPAHs. Most PAHs are found in high concentrations more than NPAHs in the atmosphere. In the atmosphere, most PAHs that have 2-4 rings (Low molecular weights, LMW) exist mainly as gases, while PAHs more than 5 rings (High molecular weight, HMW) exist in the particulate phase (Araki et al 2009; Park et al. 2002; Ravindra et al. 2006; Chang et al. 2006). In addition, most NPAHs in the atmosphere exist in gas phase or in particulate phase. Most 4 and 5 ring NPAHs exist in the particulate phase (Bamford et al. 2003). The concentration of PAHs in all sample matrices like air, water and soil is generally higher than that of NPAHs (Yaffe et al 2001).

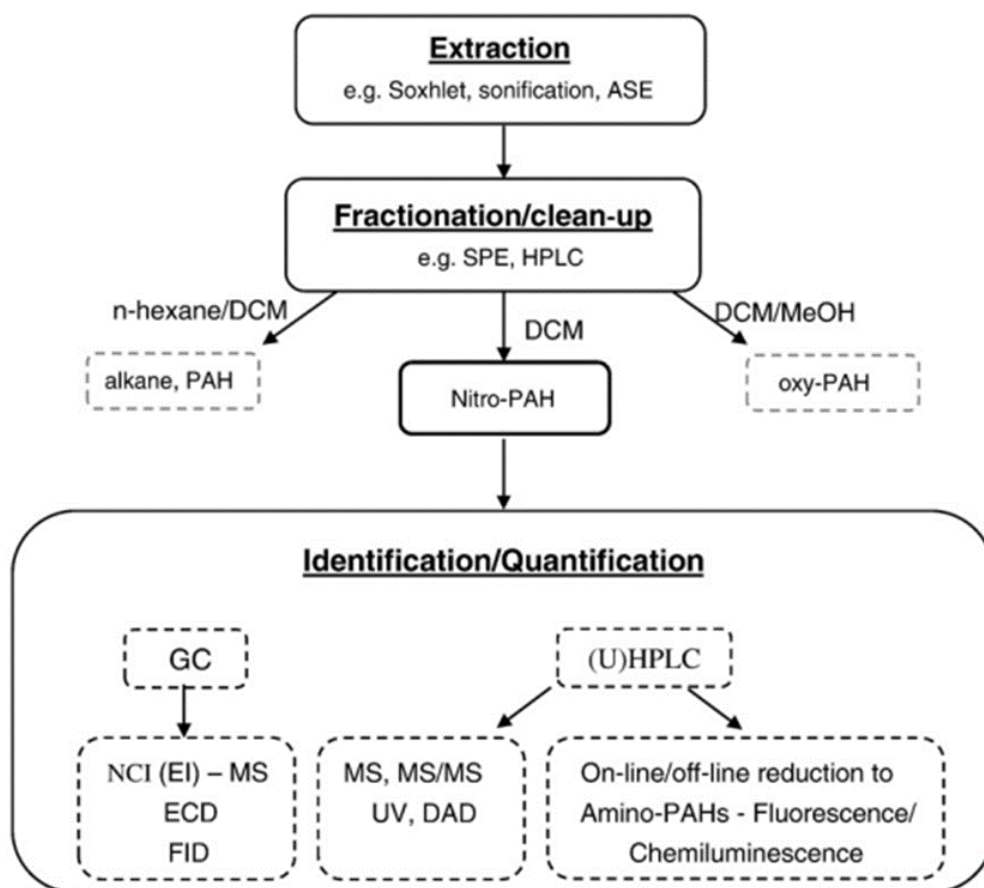


Fig 1.4 Scheme for three main steps determination of PAHs and nitro-PAHs

PAHs and NPAHs have been determined in different phases of air samples (vapor phase, total suspended particles (TSP), specific size fractions (PM₁, PM_{2.5} and PM₁₀) or size segregated fractions). The high/low volume air samplers or cascade impactors are often equipped with foams (PUFs) and/or quartz fiber filters to collect vapor phase and particulate phase PAHs and NPAHs, respectively. After sample collection, the quartz filters and PUFs are usually wrapped in aluminium foil and stored at -20 °C until analysis (Bin et al., 2007; Bandowe et al., 2017)

After sampling, air sample are need to pretreatment because complex environmental matrices and extensive cleanup of the extract prior to analysis. Bin et al. (2007) reviewed sample pretreatment techniques for PAHs in environmental matrix. Most popular solvent used for extraction are acetonitrile, benzene, dichloromethane, toluene, cyclo-hexane and also their mixture are often used as extraction solvent. Solvent extraction such as ultrasonic extraction, supercritical fluid extraction (SFE), microwave-assisted extraction, accelerat-ed/pressurized solvent extraction soxhlet extraction and concentration such as K-D evaporator and rotary evaporator concentration and clean-up with columns (Hafner and Hites, 2005) are three mostly applied pretreatment methods for PAHs samples. And subcritical water extraction has also been applied as pretreatment of air samples. On the other hand, reviewing of Bandowe et al. (2017) explained additional clean-up is usually required for NPAHs more than PAHs because of the generally lower concentration of NPAHs in environmental samples (compared to PAHs) and their lower sensitivity to detection methods.

For improving the quality of chromatographic signal and signal-to-noise ratio, interfering compounds need to be remove by cleanup steps such as solid phase extraction (SPE, with silica gel, alumina, C18, Florisil), open column chromatography (with silica gel), semi-preparative HPLC and gel permeation chromatography (GPC) have been used. In the SPE or open column technique of clean-up, the concentrated extract (of the first extraction step) is applied onto the column and eluted with solvents with different polarity. Prycek et al. (2007) studied the fractionation efficiency on a silica cartridge depending on different solvent mixtures. Applying 500 μ L of the redissolved extract in hexane on 5 g fully activated silica gel, the most effective fractionation procedure determined, was as follows: Fraction 1 (nonpolar compounds like alkanes and cycloalkanes) was eluted by 10 mL of n-hexane and then by 5 mL of n-hexane/DCM 4/1 (v/v), fraction 2 (PAHs and alkyl-PAHs) was eluted 10 mL of n-hexane/DCM 4/1 (v/v), fraction 3 (nitro-PAHs) was eluted by 10 mL of DCM, fraction 4 (oxy-PAHs, alcohols) was eluted by 10 mL of DCM/methanol (MeOH) 1/1 (v/v). Other SPE techniques use aminopropyl columns (Cochran et al., 2012), alumina and silica catridges (Albinet et al., 2006) or columns filled with silica gel (10% deactivated) (Wei et al., 2015a, 2015b). The fractionations proceed with similar combinations of solvents, starting with nonpolar solvents and ending with highly polar solvents (pentane, hexane, dichloromethane, acetone, methanol) (Wei et al., 2015a, 2015b; Albinet et al., 2014, 2006; Cochran et al., 2012). More automated fractionation techniques (normal-phase semi-preparative HPLC or GPC) have also been applied (Niederer, 1998; Watanabe et al., 1999; Hasei et al., 2006; Brorström-Lundén et al., 2010; Andrade-Eiroa et al., 2010).

A review by Bin et al. reported that the NPAHs were removed and separated from interfering substances on octadecyl-bonded silica (ODS, 4.6 \times 150mm) column as a clean-up column in the switching column technique (Bin et al., 2007). Moreover, Hayakawa and Tang have been many reviewed and reported for analyzing NPAHs were removed complicated substances by using clean-up in switching column technique (Hayakawa et al., 2001,2002, 2009, 2011,2016; Tang et al., 2003,2005, 2014, 2017). Variation method for determination PAHs and NPAHs in atmospheric have been reviewed and studied by several researchers. As earlier stated, the sample pretreatment and analytical methods for PAHs in airborne particulate (PM) are summarized and compared in Table in 1.6, It can be concluded from the table shown GC-MS with EI mode and HPLC-FL detection are the analytical methods mostly used for PAHs. Nyiri et al. has been studied and developed methods for

the quantitative determination of PAHs in PM samples. The sample preparation procedure included only a simple and quick sonication-assisted extraction step, clean-up based on addition of water and centrifugation as well as pre-concentration under N₂ stream. PM_{2.5} sampling was performed at the Széna Square, urban site in the center of Budapest, Hungary between June 2010 and May 2013. PM sample was clean-up with water. After spiking with 50 µg/L of the deuterated standard solutions, GC-MS was used for analysis of the 1PAHs (Nyiri et al. 2016). Santos et al. reported that PM samples were collected at the Lapa Bus terminal underground floor (12°58'S, 38°30'W, 52 m altitude), in Salvador, State of Bahia, Northeastern Brazil during April 27th–May 18th, 2010. PM sample were extracted with sonication and mixtures of acetonitrile (ACN) and dichloromethane (DCM) were used as solvent. No further clean-up and fractionation and 20PAHs were analyzed with GC-MS with EI mode (Santos et al., 2016). Schantz et al. studied on four particulate matters standard reference materials (SRMs) available from the National Institute of Standards and Technology (NIST) were used to evaluate the effect of solvent, number of static cycles and static times, pressure, and temperature when using pressurized liquid extraction (PLE) for the extraction of polycyclic aromatic hydrocarbons (PAHs). The four materials used in the study were SRM 1649b Urban Dust, SRM 1650b Diesel Particulate Matter, SRM 1648a Urban Particulate Matter and SRM 2975 Diesel Particulate Matter (Industrial Forklift). SRM 1649b were separated two methods (1) SRM sample extracted with dichloromethane and (2) with toluene. SRM1650b used toluene, SRM 1648 was extracted with dichloromethane and SRM2975 used two methods, SRM were extracted with only toluene and second with toluene/methanol. Finally, PAHs in SRM samples were analyzed with GC-MS with EI mode (Schantz et al., 2012). 24-h PM_{2.5} samples were collected every sixth day for 13 months from the atmosphere of a Chinese megacity. PM samples were extracted with dichloromethane as accelerated solvent, The GC/MS EI mode were determined 27PAHs (Bandowe et al., 2014).

Ding et al. were conducted each of seven sampling days, 24 hours. PM_{2.5} samples were collected using 6 stationaries as following; 2 in the kitchen, 2 in the bedroom adjacent to the kitchen, and 2 outdoors, 1–2 m above the ground and 4 personal air samplers simultaneously. PM_{2.5} were extracted by using a microwave accelerated reaction system (CEM, Mars Xpress, USA). The extracts were purified using silica/alumina chromatography. 22 target PAHs were analyzed with GC EI-MS (Ding et al., 2012). Airborne particulate matter was collected on a quartz fiber filter (8 × 10 inch², Pallflex Product, 2500QATUP) using a high-volume air sampler (HV-1000F, Shibata Co., Tokyo, Japan). Filter samples were cut into small pieces and put in a flask. The samples were then extracted 20 min under ultrasonication method with dichloromethane (DCM) and filtered, the filtrate was evaporated to dryness under a stream of N₂. The residue was dissolved in DCM 500 µL and cleaned by solid phase extraction (SPE) with a silica gel solid phase (Discovery SPE DSC-Si Silica, Supelco). The extract solution was evaporated to dryness again, and the residue was re-dissolved in 500 µL of acetonitrile. Sample solution was injected into the HPLC-FLD system (Kojima et al., 2010). Tang et al. studied and determined PAHs in airborne particulates which were collected in winter and summer from 1997 to 2002 in seven cities in the Pan–Japan Sea countries, Shenyang (China), Vladivostok (Russia), Seoul (South Korea), Kitakyushu, Kanazawa, Tokyo and Sapporo (Japan). A piece of each filter of airborne particulates was cut into small pieces in a flask and 9PAHs compound were extracted with ultrasonication twice with benzene/ethanol (3/1), particulates were analyzed by HPLC with fluorescence (Tang et al., 2005). PM in indoor and outdoor were determined PAHs in summer

and winter at two industrial cities in Shizuoka, Japan, by Ohura et al. reported that airborne particles were collected and size-fractionated using a sampler that combined a minipump (MP-603 T or MP-Σ300; Sibata Scientific Technology Ltd., Tokyo, Japan) with a three-stage cascade impactor (Tokyo Dylec Corp., Tokyo, Japan); the flow rate for sampling was 3.0 l min^{-1} . Airborne particles were collected and monitored of fractionated particles in indoor and outdoor air was conducted in the cities of Fuji and Shimizu, two proximate (ca. 30 km) cities in Shizuoka Prefecture. Monitoring of 18 PAHs in particles phase were extracted with DCM by using an ultrasonic, centrifugation at 3000 rpm for 10 min and evaporation under N_2 . PAHs were analyzed with HPLC-FL detection (Oharu et al., 2004).

Table 1.6 Analytical performance of previous methods for PAHs

Analytical instruments	Extraction method	PAHs analyzed	Limits of detection (LOQs)
GC-EI-MS (Nyiri et al, 2016)	QuEChERS	16	42 pg/m^3
GC-EI-MS (Santos et al, 2016)	Sonication-assisted	20	$0.28\text{-}1.1 \text{ pg/m}^3$
GC/EI-MS (Bandowe et al, 2014)	ASE	27	Not validated
GC/EI-MS (Ding et al, 2012)	Microwave-assisted	22	$92\text{-}229 \text{ ng mL}^{-1}$
GC/EI-MS (Schantz et al, 2012)	PLE	16	Not validated
HPLC-FL (Kojima et al, 2010)	Sonication-assisted	7	Not validated
HPLC-FL (Oharu et al, 2004)	Ultrasonication with DCM	18	Not validated
HPLC-FL (Tang et al, 2005)	Ultrasonication with benzene/ethanol	9	Not validated

The determination of NPAHs have been reviewed by Bandowe et al., NPAHs extracted from airborne particulate matters reports have dealt with NPAH concentrations in air, mostly due to their low concentrations and difficulty in detection. Several methods have been used for analysis of NPAHs in atmospheric environment, such as NPAHs have been analyzed by gas chromatographic

(GC) and liquid chromatographic (LC) methods. These include GC with NCI (negative chemical ionization)-MS, The most common detection technique for the analysis of nitro-PAHs with HPLC is fluorescence (Watanabe et al., 1999; Hasei et al., 2006; Schauer et al., 2004; Garcia-Alonso et al., 2012; Lung and Liu, 2015). Because of the high electron-withdrawing nitro-group. Nitro-PAHs are not fluorescent and need to be reduced to their amino derivatives, which are strongly fluorescent. Nitro-reduction can be performed before the HPLC (off-line methods) or directly in a chromatographic system (on-line methods). Off-line methods are time consuming and difficult to automate for routine analysis. On-line methods need more complicated HPLC equipment including a reduction column. Off-line reduction procedures include the use of reagents such as sodium tetrahydroborate with copper(II)chloride ($\text{NaBH}_4\text{-CuCl}_2$), sodium hydrosulfide, and zinc powder in hydrochloric acid (Garcia-Alonso et al., 2012; Zielinska and Samy, 2006). Previously used on-line reduction procedures include Pt/Rh-coated Al_2O_3 , Pt coated Al_2O_3 and Zn/glass bead packed in columns (Zielinska and Samy, 2006; Delhomme et al., 2007). Another sensitive detection technique is chemiluminescence. First the nitro-PAHs have also to be reduced to amino-derivatives which then react with a mixture of bis(2,4,6- trichlorophenyl)oxalate (or TCPO) and hydrogen peroxide (or H_2O_2) and form an excited state of the amino-compounds (Cvacka et al., 1998; Zielinska and Samy, 2006). The advantages and disadvantages of these detection methods are elaborated in previous review paper (Zielinska and Samy, 2006). For quality assurance purposes, we recommend the simultaneous determination of nitro-PAHs in certified reference materials (if available) or spiked samples using the same analytical procedure as being used for nitro-PAH determination in the real samples.

The use of GC-NICI-MS have allowed for quantification of several NPAHs in airborne particulates, Nyiri et al. has been developed for the quantitative determination of NPAHs in PM samples. The sample preparation procedure included only a simple and quick sonication-assisted extraction step, clean-up based on addition of water and centrifugation as well as pre-concentration under N_2 stream. $\text{PM}_{2.5}$ sampling was performed at the Széna Square, urban site in the center of Budapest, Hungary between June 2010 and May 2013. PM sample was clean-up with water. After spiking with 50 $\mu\text{g/L}$ of the deuterated standard solutions, GC-MS with NICI mode was used for analysis of the 11 NPAHs (Nyiri et al. 2016).

Albit et al. has been developed an extraction procedure based on the Quick EasyCheap Effective Rugged and Safe (QuEChERS) and used for analysis of particle-bound nitrated. This analytical procedure enabled simultaneous GC–NICI-MS quantification of 32 NPAH and 32 OPAH (Albinet et al., 2014). Bandowe et al. were collected $\text{PM}_{2.5}$ samples 24h, every sixth day for 13 months from the atmosphere of a Chinese megacity. PM samples were extracted with dichloromethane as accelerated solvent, The GC/MS NICI mode were determined 14NPAHs (Bandowe et al., 2014). Wang et al. was collected $\text{PM}_{2.5}$ at Xujiahui and Baoshan in Shanghai, $\text{PM}_{2.5}$ sample were selected 48 samples and spiked with appropriate amounts of recovery standards then extracted with DCM and applied to silica gel/ Al_2O_3 chromatography in order to remove macromolecules and polar interfering compounds. Eluate with hexane: dichloromethane (1:1) was collected. 8NPAHs were analyzed with GC/MS NICI mode (Wang et al., 2014). Particle phase in ambient air were collected in downtown Baltimore, MD, an urban region, and in Fort Meade, MD, PM samples were extracted with soxhlet extraction. The GC-NICI-MS was analyzed 26 NPAHs in PM samples. Ding et al. were reported

concentration of 12 nitro-PAHs which were analyzed from particulate samples. PM sample were collected 24 h, for 7 sampling days with using 6 stationaries. The particulate phase of PM_{2.5} were extracted with 25 ml of the same mixture using a microwave accelerated reaction system (CEM, Mars Xpress, USA). The extracts were purified using silica/alumina chromatography. 22 target PAHs were analyzed with GC NICI-MS (Ding et al., 2012).

Kojima et al. was collected PM on quartz fiber filter by using high-volume air sample (Kimoto Electric Company Ltd., Osaka, Japan) with a flow rate of 0.5 or 1.1 m³/min for 24 hours. PM samples were collected on the rooftop. PM₁₀ samples were extracted with DCM by using ultrasonication. Nitro-PAHs analysis, the in-line reduction with HPLC-CL detection method was employed. The chemiluminescence reagent solution was 8 mM of H₂O₂ and 0.64 mM of TCPO in acetonitrile (Kojima et al., 2010). Tang et al. improved HPLC-CL detection for determined 21 NPAHs in airborne particulate which were collected at a heavy traffic site in Kanazawa, Japan by a 123VL high-volume air sample (Kimoto Electric Company Ltd., Osaka, Japan) with a 2500QAT-UP quartz fiber filter with a flow rate of 1.3 m³/min for 24 hours. PM sample were extracted with ultrasonication method by two organic solvent (benzene and methanol). Clean-up column was applied to switching column sytem. 21 NPAHs were separated and quantified with HPLC-CL detection. For separation of NPAHs, nitro-PAHs have to reduce to corresponding amino-derivatives which then react with a mixture of TCPO and H₂O₂ and acetonitrile in the switching system. NPAHs in atmospheric particulate matters also have been studied and reported with HPLC fluorescence or chemiluminescence. Schauer et al. studied on samples of fine air particulate matter (PM_{2.5}). The PM_{2.5} sample was extracted with 30 mL DCM / MeOH / toluene (1:1:1; v/v/v) for 30 min in an ultrasonic bath (Sonorex, Berlin, Germany). The filter aliquot was removed and the extract was directly transferred into a tapered flask (50 ml, Roth, Karlsruhe, Germany) and evaporated to ~0.5 mL. The nitro-PAH fraction residue was dissolved in 200 µL cyclohexane and measured by HPLC-Reduction-Fluorescence (Schauer et al., 2004).

Table 1.7 Analytical performance of previous methods for NPAHs

Analytical instruments	Extraction method	NPAHs analysed	Limits of detection (LODs)
GC-NICI-MS (Nyiri et al, 2016)	QuEChERS	11	83 – 167 pg/m ³
GC/NICI-MS (Wang et al, 2014)	n.a., DCM	8	1.34-6.01 pg/m ³
GC/NICI-MS (Bandowe et al, 2014)	ASE	9	Not validated
GC/NICI-MS (Albinet et al, 2014)	QuEChERS	32	Not validated
GC/NICI-MS (Ding et al, 2012)	Microwave-assisted	12	26-450 ng mL ⁻¹
GC/NICI-MS (Bamford et al, 2003)	Soxhlet	26	1-990 pg/m ³
HPLC/CLD (Kojima et al, 2010)	Ultrasonication	2	Not validated
HPLC/CLD (Tang et al, 2005)	Ultrasonication	21	0.3-210 pg/injection
HPLC/FLD (Schauer et al, 2004)	Ultrasonication	12	6-100 pg

1.7 Motivation of the dissertation

PAHs and their derivatives are one of the typical air pollutants mentioned above and the importance of them in the adverse health effects of PM has been well studied. Airborne PM has been reported about their carcinogenic activities by the International Agency for Research on Cancer (IARC) (IARC 2016) and IARC has classified PM as carcinogenic to human (Group 1) and has also categorized various PAHs and NPAHs as Group 1, Group 2A (probably carcinogenic to humans) or Group 2B (possibly carcinogenic to humans) (IARC 2016). Emission sources, formation, concentrations and characteristic of atmospheric PAHs and NPAHs have been reported by many researches. PAHs and NPAHs are almost emitted by incomplete combustion processes such as, residential heating, vehicle exhaust, industries product process coal and wood burning and natural sources. Atmospheric NPAHs are produced by not only primary sources such as diesel engine exhaust, but also secondary reactions of their parent PAHs in the atmosphere. In particular, NPAHs found in the atmosphere lower concentrations than their parent PAHs therefore, considerable effort has been expended to determine NPAHs in environmental samples. Numerous studies focused on only the quantitative determination of PAHs in PM samples and 16 priority PAHs listed by U.S. Environmental Protection Agency (EPA) have been generally determined and discussed. On the other hand, the data analysis between PAH and NPAH concentrations is useful for the source

identification. For example, 1-nitropyrene (1-NP) is an important marker for automobile exhaust and the ratio of 1-NP to PAHs has been used to discuss the contribution of the exhaust in urban PM samples. Several NPAHs formed in the atmosphere via reactions of their parent PAHs with OH or NO₃ radicals have been used as markers for atmospheric reactions (Jariyasopit et al 2014). The analysis of both PAHs and NPAHs is essential to discuss sources and health effects of airborne PM such as PM_{2.5}.

Many methods for the determination of PAHs and NPAHs in the atmospheric PM have been developed. Mostly, PAHs are analyzed by GC-EI-MS and HPLC-FL methods (Bin et al., 2007). NPAHs have been determined by using GC-NICI-MS, HPLC-FL and HPLC-CL methods (Zielinska et al., 2006; Bandown et al., 2017). Although most of the earlier works have been successfully determined PAHs and NPAHs in PM samples, there are some limitations for simultaneous determination of PAHs and NPAHs. For example, GC-EI-MS is a powerful tool for identifying each analyte, however PAH isomers with the same monitoring ions which were separated on a column. Benzo[*k*]fluoranthene (BkF) and benzo[*b*]fluoranthene (BbF) are a representative combination of PAHs that are difficult to separate (Quintas et al 2008). Although GC-NICI-MS has been applied to detect NPAHs, PAHs are not detectable by the NICI mode (Bandown et al., 2014; Keyte et al 2016). HPLC methods for NPAHs entailed the reduction of the CL detection have been successfully applied to their analysis. However, The CL system was complicated by the extra equipment needed for post-column CL reagents, which consume an especially large amount of acetonitrile. Furthermore, the CL detection cannot be applied to PAH analysis because the fluorescence characteristics of PAHs are inadequate for sensitive detection (Hayakawa et al., 2016a). At present, FL detection of NPAHs should be more sensitive and can save solvent compared to CL detection. The HPLC-based method also enables a large-scale injection (100 µL or more), simultaneous analysis of PAHs and NPAHs, and can be used for the analysis of samples with a small sampling volume limited by collecting instruments such as personal samplers.

Therefore, a study on development of HPLC-FL detection for simultaneously determination PAHs and NPAHs in the atmospheric PM is important and it would provide progress in their analytical method. Characteristics of reversed phase columns to effectively purify and collect PAHs and NPAHs fraction in sample matrix was examined. A two-dimensional HPLC method consisting of the clean-up, reduction, column switching, separation and FL detection steps was developed for target 10 PAHs and 18 NPAHs. The structures of target 10 PAHs and 18 NPAHs are shown in Fig. 1.5. The developed on-line system makes it possible to apply the crude extract of real samples without any complicated pretreatment before injection. The performance and potential of the developed method were validated in the presence of sample matrix using actual PM_{2.5} samples and the standard reference material (SRM1649b, urban dust).

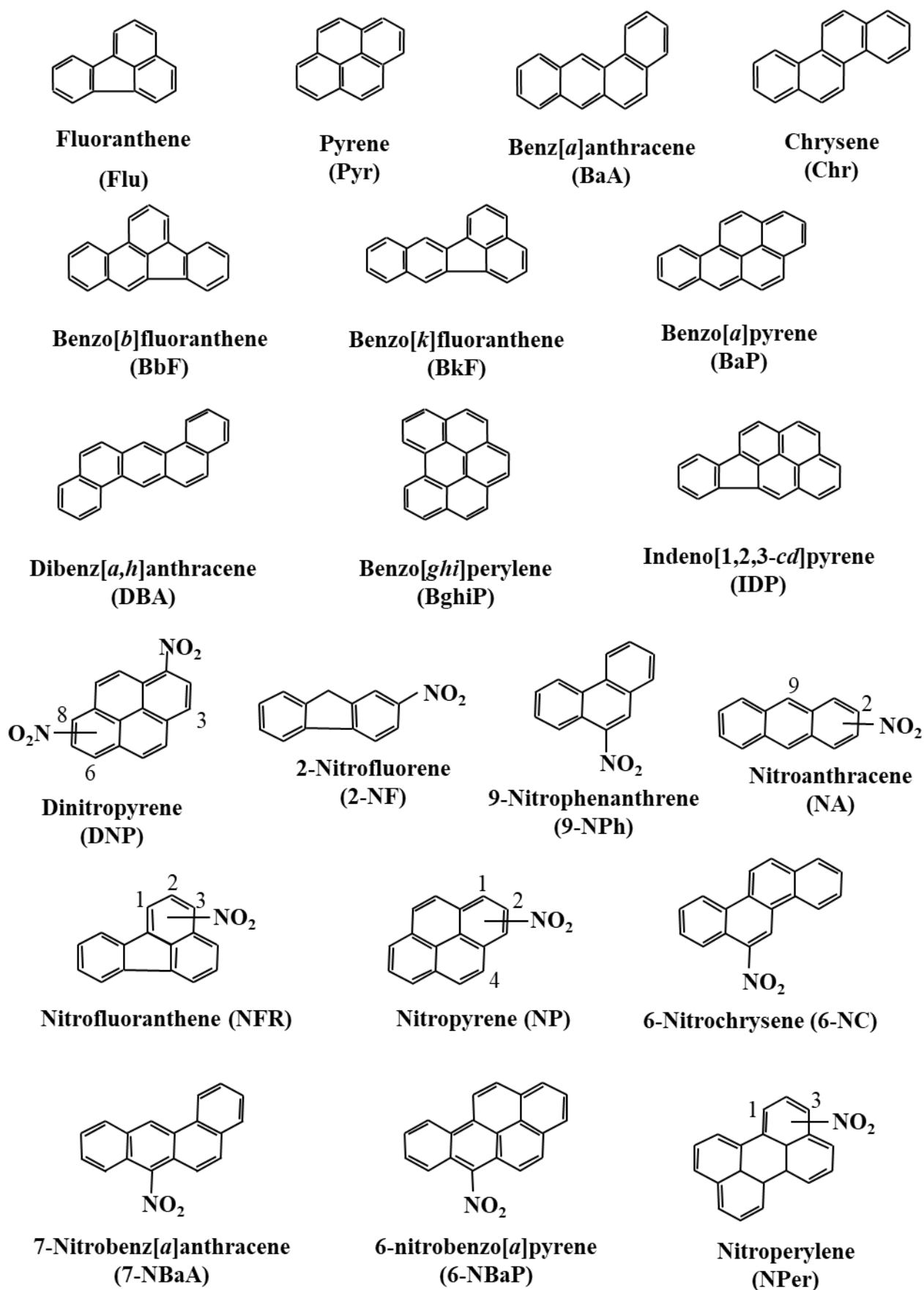


Fig. 1.5 Structures of the 10 PAHs and 18 NPAHs analyzed in this study

Chapter 2

Development of an HPLC-fluorescence method for polycyclic aromatic hydrocarbon and their Nitro derivatives in atmospheric particulate matter

2.1 Introduction

Airborne particulate matter (PM) is produced by the combustion of organic materials and from atmospheric gaseous reactions. Airborne PM has been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (IARC 2016). Polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs) are hazardous chemicals commonly found in PM, with many of these compounds having potential carcinogenic and/or mutagenic properties (Choi et al., 2010; IARC 2013; IARC 2016). The IARC has categorized several PAHs and NPAHs such as benzo[*a*]pyrene (BaP) as Group 1 (carcinogenic to humans), 1-nitropyrene (1-NP) as Group 2A (probably carcinogenic to humans) and several other PAHs and NPAHs as Group 2B (possibly carcinogenic to humans) (IARC 2016). Additionally, NPAHs are of specific concern because dinitropyrenes (DNPs) have been shown to exhibit the strongest direct mutagenic effects of this class of compounds (Bandowe and Meusel, 2017). The presence of NPAHs in the ambient atmosphere has been shown to contribute to the mutagenicity of PM (Hayakawa et al., 1995).

PAHs and NPAHs are mostly emitted by incomplete combustion processes from residential heating, vehicle exhaust, and coal and wood burning (Tang et al., 2009; IARC 2013; Chuesaard et al., 2014; Cheruiyot et al, 2015; Bandowe and Meusel, 2017). Atmospheric NPAHs are produced not only by primary sources such as diesel engine exhaust, but also secondary reactions of their parent PAHs in the atmosphere (Bandowe and Meusel, 2017). NPAHs are generally found in the atmosphere at very low concentrations (several pg/m^3 ~ in total) which are about 1 - 3 orders of magnitude lower than their parent PAHs (Bandowe and Meusel, 2017). Therefore, considerable efforts have been expended to determine NPAHs in environmental samples (Cvačka et al., 1998; Hayakawa et al., 2016a, 2017). Numerous studies focused on only the quantitative determination of PAHs in PM samples and, specifically, the 16 priority PAHs listed by U.S. Environmental Protection Agency (EPA) have been widely determined and discussed (Cheruiyot et al, 2015). However, analysis of interrelationship between PAH and NPAH concentrations as well as individual components is useful for source identification. For example, 1-NP is an important marker for automobile exhaust and the ratio of 1-NP to PAHs has been used to determine the contribution of car exhaust to urban PM samples (Tang et al., 2009; Chuesaard et al., 2014; Hayakawa et al., 2016a). Murahashi et al. (1994) reported an HPLC method for simultaneously determining PAHs and NPAHs with one injection. However, two independent flow passes were required to detect PAHs and NPAHs with FL and CL detectors, respectively. Several NPAHs formed in the atmosphere via reactions of their parent PAHs with OH or NO₃ radicals have been used as markers for atmospheric reactions (Ciccicoli et al., 1996; Jariyasopit et al., 2014; Tang et al., 2014; Bandowe and Meusel, 2017). The analysis of both PAHs and NPAHs is essential to discuss sources and health effects of airborne PM such as PM_{2.5}.

Currently, PAHs in the environmental matrix are mostly analyzed by gas chromatography-mass spectrometry (GC-MS) with electron impact ionization (EI) mode (Bandowe et al., 2014; Nyiri et al., 2016; Ma et al., 2016; Hayakawa et al., 2017), and high-performance liquid chromatography with fluorescence detection (HPLC-FL) (Toriba et al., 2003; Hayakawa et al., 2016a, 2017). Although the selected ion monitoring of GC-MS is a powerful tool for identifying each analyte, PAH isomers with the same monitoring ions should be separated on a column. Benzo[*k*]fluoranthene (BkF) and benzo[*b*]fluoranthene (BbF) are representative of PAHs that are difficult to separate using GC-MS (Quintas et al. 2008). HPLC-FL provides an alternative method for the quantification of PAHs in environmental PM samples because of the high fluorescence quantum yield and detection specificity of PAHs and good separations of the isomers on reversed-phase columns such as polymeric-type octadecyl-bonded silica (ODS) columns (Toriba et al., 2003).

GC-MS(/MS) methods for determining atmospheric NPAHs are typically accompanied by detection with negative ion chemical ionization (NICI) mode (Kawanaka et al., 2007; Bandowe et al., 2014; Albinet et al., 2014). However, it is difficult to simultaneously analyze NPAHs and PAHs because PAHs are not detectable in NICI mode (Bandowe et al., 2014; Keyte et al., 2016). HPLC methods for analysis of NPAHs entail reduction of the analytes, separation by reversed phase columns and detection via fluorescence or chemiluminescence (HPLC-FL or HPLC-CL) (Cvačka et al., 1998; Tang et al., 2003, 2005a; Ohno et al., 2009). In typical HPLC protocols, manual reduction of NPAHs to their corresponding amino-derivatives can be performed (Kamiura et al., 1991; Hayakawa et al., 1992, 1995) or reduction can be achieved through a column packed with platinum/rhodium (Pt/Rh)-coated alumina (Tang et al., 2003, 2005a; Ohno et al., 2009; Hayakawa et al., 2016a, 2017). Reduction is required to obtain molecules with fluorescent properties. A reduction column provides efficient reduction of NPAHs and is commonly applied to HPLC-based methods for analysis of NPAHs. The resulting amino-derivatives can be separated on reversed-phase column(s) and then detected by an FL detector or a CL detector after the reaction with peroxy oxalate esters such as (2,4,6-trichlorophenyl) oxalate and hydrogen peroxide as post-column reagents (Cvačka et al., 1998; Hayakawa et al., 2016a, 2017). While FL detectors suffered from poor performance for the detection sensitivity for NPAHs in PM samples, CL detection has been successfully used for their analysis. However, the CL system is complicated by the extra equipment needed for post-column CL reagents, which consume an especially large amount of acetonitrile. Furthermore, the CL detection cannot be applied to PAH analysis because the fluorescence characteristics of PAHs are inadequate for sensitive detection (Hayakawa et al., 2016a). The sensitivity of FL detectors has improved remarkably over the last 20 years. At present, FL detection of NPAHs is more sensitive and saves solvent compared to CL detection. The HPLC-based method also enables a large-scale injection (100 μ L or more), simultaneous analysis of PAHs and NPAHs, and can be used for the analysis of samples with a small sampling volume limited by collecting instruments such as personal samplers.

The aim of this study is to develop an HPLC system for simultaneous determination of PAHs and NPAHs using only a FL detector based on our previous HPLC-CL method. At the same time, this method simplifies the HPLC system, shortens the total analytical time and reduces solvent consumption. First, we examined characteristics of reversed phase columns to effectively remove sample matrix and collect PAH and NPAH fractions. A two-dimensional HPLC method consisting of clean-up, reduction, column-switching, separation and FL detection steps was developed for determining 10 PAHs and 18 NPAHs in airborne particulates such as PM_{2.5}. The developed system allows for the application of crude extract of environmental samples without any complicated

pretreatment before injection. The performance and potential of the method were validated using actual PM_{2.5} samples and standard reference material (SRM1649b, urban dust).

2.2 Materials and methods

2.2.1 Chemicals and reagents

The USEPA 610 PAHs mix, a mixture of 10 PAHs (10 µg/mL in acetonitrile) including fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DBA), benzo[*ghi*]perylene (BghiP) and indeno[1,2,3-*cd*]pyrene (IDP) was purchased from Sigma-Aldrich (St.Louis, MO, USA). Three internal standards for PAHs, pyrene-*d*₁₀ (Pyr-*d*₁₀), benzo[*a*]anthracene-*d*₁₂ (BaA-*d*₁₂) and benzo[*a*]pyrene-*d*₁₂ (BaP-*d*₁₂) were purchased from Wako Pure Chemicals (Osaka, Japan). 1,6-Dinitropyrene (1,6-DNP), 1,3-dinitropyrene (1,3-DNP), 1,8-dinitropyrene (1,8-DNP), 2-nitroanthracene (2-NA), 9-nitroanthracene (9-NA), 9-nitrophenanthrene (9-NPh), 2-nitrofluorene (2-NF), 2-nitrofluoranthrene (2-NFR), 3-nitrofluoranthrene (3-NFR), 1-nitropyrene (1-NP), 7-nitrobenz[*a*]anthracene (7-NBaA), 6-nitrochrysene (6-NC), 6-nitrobenz[*a*]pyrene (6-NBaP) were purchased from AccuStandard, Inc. (New Haven, CT, USA) (100 µg/mL in toluene). 1-Nitrofluoranthrene (1-NFR), 2-nitropyrene (2-NP), 1-nitroperylene (1-NPer) and 3-nitroperylene (3-NPer) were supplied from Chiron AS (Trondheim, Norway)) (0.1 mg/mL in toluene), and 4-nitropyrene (4-NP) was from Tokyo Chemical Industry (Tokyo, Japan). 6-Nitrochrysene-*d*₁₁ (6NC-*d*₁₁) for the internal standard of NPAH analysis was purchased from Cambridge Isotope Lab. Inc. (Andover, MA, USA). All solvents and other chemicals were used of analytical-reagent or HPLC grade. Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). The Standard reference material of urban dust (SRM1649b) was purchased from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The SRM sample is an atmospheric particulate material collected in an urban site and certified concentrations of some PAHs and NPAHs have been reported (Schantz et al., 2012; Albinet et al., 2014; NIST 2016).

2.2.2 Column evaluation for clean-up column

For column evaluation, a simple HPLC-FL system (Fig. 2.1) was used to confirm the retention times of PAHs and NPAHs on the candidates of the clean-up columns. The system consisted of two HPLC pumps (LC10A pumps) for gradient elution, two column ovens (CTO-10AS) (all from Shimadzu) for the tested columns and the reduction column (NPPak-RS, 10×4.6 mm i.d., JASCO, Tokyo, Japan) and an auto sample injector (SIL-10A), a fluorescence detector (RF-10A), a system controller (SCL-10A), a degasser (DGU-14A). Eight reversed-phase columns, 2 octadecyl (5C₁₈-MS-II and 5C₁₈-AR-II)-, phenylethyl (PE-MS)-, pentafluorophenyl (PFP)-, nitrophenylethyl (NPE)-, naphthylethyl (πNAP)-, pyrenylethyl (PYE)- and pentabromobenzyl (PBr)-bonded silica columns (Cosmosil columns, 150×4.6 mm i.d., 5µm, all from Nacalai tesque, Kyoto, Japan) were examined (Table 2.1). A gradient elution using water (eluent A) and methanol (eluent B) was carried out (B, 70-100% liner gradient for 60 min, 100% isocratic after 60 min) at a flow rate of 1.0 mL/min. To evaluate the performance of column characteristics, the retention factor (*k*) was considered to be a factor for parameter calculation. A parameter, the switching index is defined as shown in Eq. (1)

$$\text{Switching index} = (k_{\max} - k_{\min}) / k_{\text{mean}} \quad (1)$$

where k_{\max} , k_{\min} and k_{mean} are the maximum, minimum and mean of retention factors (k) of all target compounds, respectively. The overlap and length of elution times for each column was evaluated.

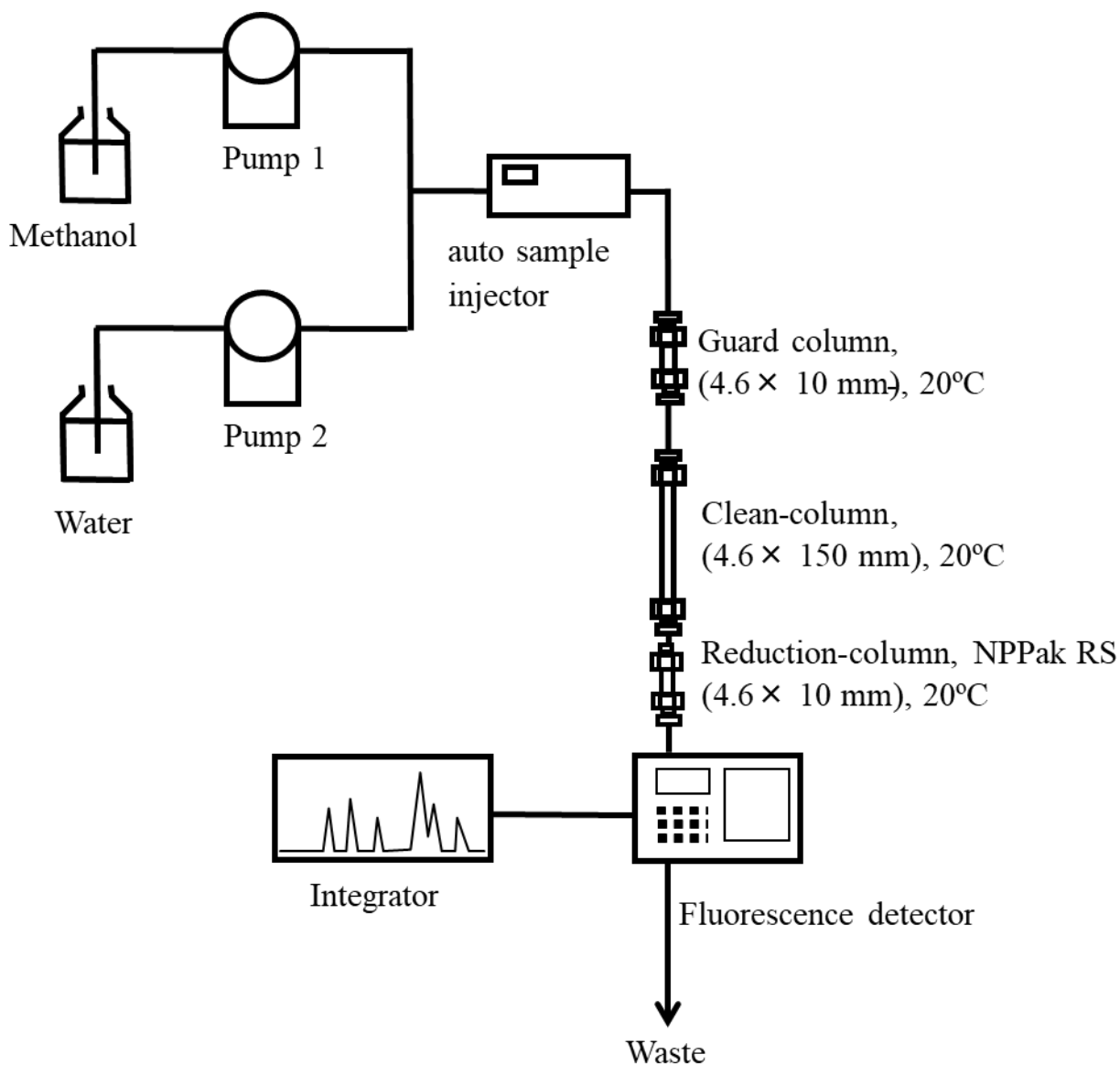


Fig. 2.1 Schematic diagram of the simple HPLC-FL method for column evaluation

Table 2.1 Major interaction of the examined columns

Stationary phase	Abbreviation	Major interaction
Octadecyl group (monomeric type)	5C ₁₈ -MS-II	Hydrophobic interaction
Octadecyl group (polymeric type)	5C ₁₈ -AR-II	Hydrophobic interaction
Phenylethyl group	PE	Hydrophobic and π - π Interactions
Nitrophenylethyl group	NPE	Hydrophobic, π - π and dipole-dipole Interactions
Pentafluorophenyl group	PFP	Hydrophobic, π - π and dipole-dipole Interactions
Naphtylethyl group	π Nap	Hydrophobic and π - π Interactions
Pyrenylethyl group	PYE	Hydrophobic, π - π and dispersion interactions
Pentabromobenzyl group	PBr	Hydrophobic and dispersion interactions

2.2.3 HPLC system and conditions

A schematic diagram of the HPLC-FL system used for the simultaneous determination of PAHs and NPAHs is shown in Fig. 2.2. The system consists of 4 LC20AD pumps (Pump 1-4), a SIL-20AC auto sample injector, a degasser (DGU-20A5), a CMB-20A system controller and an integrator (LCsolution software), a CTO-20 AC column oven, a six-port switching valve, and a RF-20Axs fluorescence detector (All from Shimadzu, Kyoto, Japan). The injected sample was eluted through a clean-up column (Cosmosil, 5NPE, 150 × 4.6 mm i.d., 5 μ m, Nacalai Tesque, Kyoto, Japan) with its guard column 1 (10 × 4.6 mm i.d.) and then NPAHs were reduced to their amino-derivatives by using a reduction column (NPpak-RS, 10 × 4.6 mm i.d., JASCO, Tokyo, Japan) at 80°C (0 - 15.4 min, switching valve position A). The mobile phase for the clean-up and reduction columns was ethanol/acetate buffer (pH5.5) (95/5, v/v) at a flow rate of 0.2 mL/min. A fraction of the amino-derivatives and unchanged PAHs eluted from the reduction column with the mobile phase was mixed with 30 mM ascorbic acid through the guard column 2 (Asahipak ODP-50G-6A, 10 × 6.0 mm i.d., 5 μ m, Shodex, Tokyo, Japan) at a flow rate of 1.6 mL/min and was then trapped on the concentration column (Spheri-5 RP-18, 30 × 4.6 mm i.d. 5 μ m, Perkin Elmer, MA, USA) with a switching time of 15.4-33.0 min (position B). The concentrated fraction was passed through two separation columns (Inertsil ODS-P, 250 × 4.6 mm i.d., 5 μ m, GL Sciences, Tokyo, Japan) with their guard column 3 (10 × 4.6 mm i.d.) in tandem (33.0 - 120.0 min, position A).

All columns except the reduction column were maintained at 20°C. A gradient elution of the separation columns was performed using 10 mM imidazole buffer (pH 7.6) as eluent A and

acetonitrile as eluent B. The gradient conditions (B concentration and flow rate) for the separation of amino-derivatives of NPAHs and unchanged PAHs are presented in Table 2.2. Finally, the separated analytes were detected with their optimum excitation (Ex.) and emission (Em.) wavelengths by the dual-channel FL detector. Excitation and emission wavelengths used for PAHs and the reduced NPAHs are given in Table 2.3.

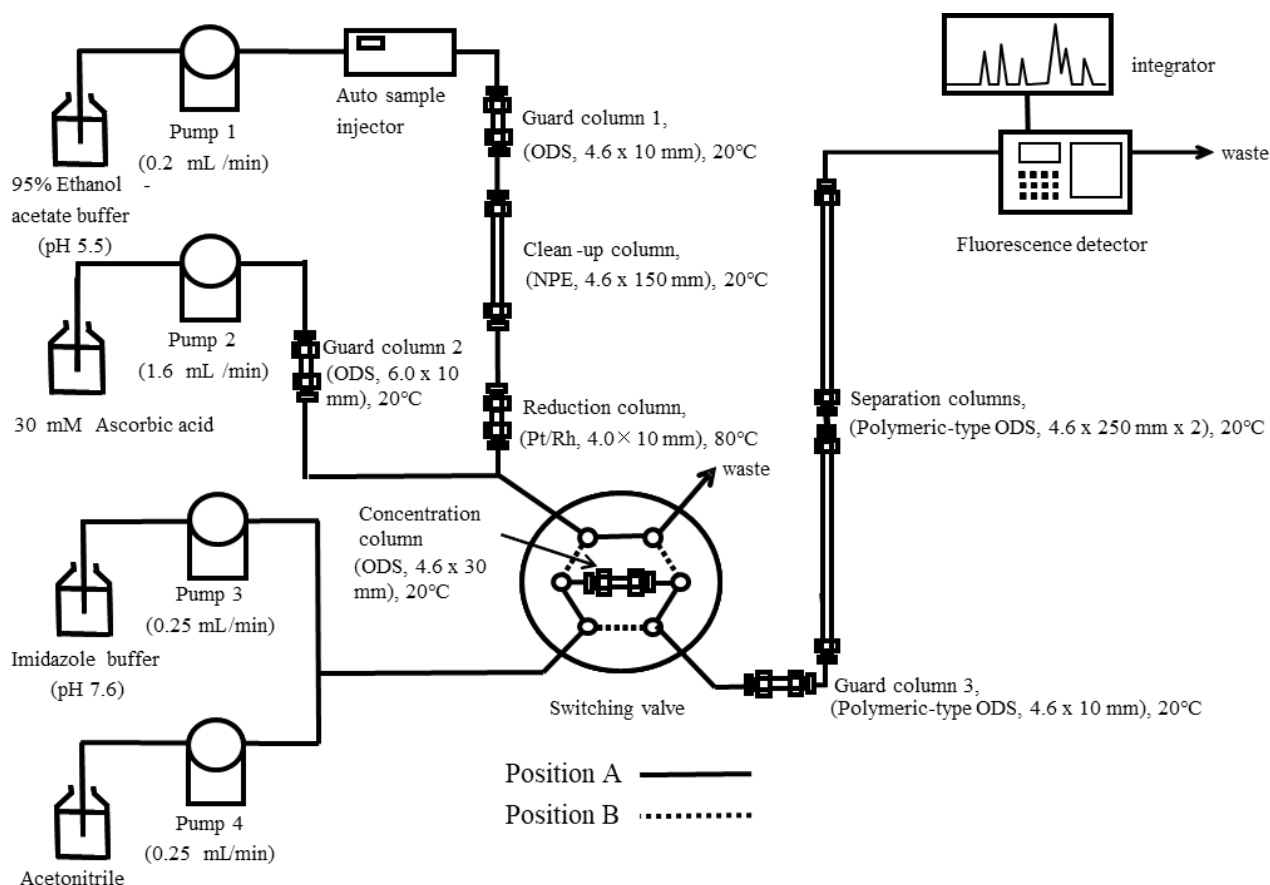


Fig. 2.2 Schematic diagram of the developed HPLC-FL method

Table 2.2 Gradient conditions for the separation of amino derivatives of NPAHs and unchanged PAHs

Acetonitrile concentration		Flow rate	
Time (min)	B (%) ^{a)}	Time	Total flow (mL/min)
0–15.40	20	0–15.40	0.5
15.40–33.00	65	15.40–33.00	0.5–0.8 ^{c)}
33.00–65.50	80	33.00–66.50	0.8
66.50–86.50	100	66.50–86.50	1
86.50–117.00	20 ^{b)}	86.50–117.00	1.8 ^{c)}

^{a)} The concentration varied using the stepwise gradient mode.

^{b)} Initializing process after analysis.

^{c)} The flow rate increased linearly with time.

Table 2.3 Time program for excitation and emission wavelengths

Compound	Time (min)	Excitation wavelength (nm)	Emission wavelength (nm)	Channels
PAHs				
Flu	78.0-80.7	289	450	2
Pyr	80.3-82.7	331	391	1
Chr	88.7-91.0	265	381	1
BaA	86.0-112.6	264	407	2
BbF	91.0-103.5	295	420	2
BkF	91.0-103.5	295	420	2
BaP	86.0-112.6	264	407	2
DBA	103.5-120.0	286	397	1
BghiP	86.0-112.6	264	407	2
IDP	112.6-120.0	300	530	2
NPAHs				
1,6-DNP	0-52.5	369	442	1
1,3-DNP	0-52.5	395	454	2
1,8-DNP	0-52.5	395	454	2
2NF	52.5-56.2	285	370	2
9NPh	56.1-59.2	345	430	1
9NA	56.1-59.2	260	490	1
2NA	59.2-63.2	265	488	1
1NFR	56.2-63.0	273	437	2
2NFR	59.2-63.2	265	488	1
3NFR	63.0-65.5	300	530	2
4NP	63.2-66.5	360	430	1
1NP	63.2-66.5	360	430	1
2NP	66.5-78.0	338	438	1
	66.5-78.0	273	437	2
7NBaA	69.5-75.2	300	475	1
6NC	66.5-78.0	273	437	2
1NPer	75.2-80.3	227	540	1
6NBaP	82.7-86.4	420	475	1
	82.7-86.0	283	513	2
3NPer	84.3-88.7	227	540	1
Internal standards				
Pyr-d ₁₀	80.3-82.7	331	391	1
BaA-d ₁₂	86.0-112.6	264	407	2
BaP-d ₁₂	86.0-112.6	264	407	2
6NC-d ₁₁	66.5-78.0	273	437	2

2.2.4 Particulate matter samples and extraction procedure

PM_{2.5} samples were collected on a quartz fiber filter (2500QAT-UP, Pall Life Sciences, Ann Arbor, MI, USA) by a high-volume air sampler (Model HV-700F, Shibata Sci. Tech., Saitama, Japan) with an impaction plate for a 50% cutoff point of 2.5 µm (PM_{2.5}) for 24 h at a flow rate of 1000 L/min. The PM_{2.5} samples were collected at an urban site in Kanazawa, Japan from November 5 to 17, 2016 and they were analyzed to determine the accuracy and precision of the developed method. The filters were stored at -20°C until analysis. The commercially available urban dust (SRM 1649b) was from atmospheric particulate material collected in the Washington, DC area in 1976 and 1977 (NIST 2016). After the addition of internal standards, a mixture of Pyr-*d*₁₀, BaA-*d*₁₂ and BaP-*d*₁₂ (25, 12 and 13 ng, respectively) for PAH quantification and 6-NC-*d*₁₁ (1.8 pg) for NPAH quantification, the samples were ultrasonically extracted with dichloromethane (DCM) for 15 min. One-fourth of the PM_{2.5} filters was cut into small pieces and extracted with 75 mL of DCM. The SRM samples (3 mg of the powder) were extracted with 5 mL of DCM. The extraction procedure was repeated 3 times. After adding 30 µL of dimethylsulfoxide (DMSO) to the extract, the DCM in the extract was completely evaporated. The resulting DMSO solution was mixed with 270 µL of ethanol. Finally, the solution was filtered through a centrifugal filter (Ultrafree-MC, 0.45 µm Millipore) and then an aliquot (100 µL) of the solution was injected into the developed HPLC-FL system.

2.2.5 Calibration, sensitivity, accuracy and precision

The developed method was validated with calibration curves, the limit of detection (LOD), the limit of quantification (LOQ), precision and accuracy. The concentrations of PAHs and NPAHs were quantified from the peak area ratios of the analytes to the deuterated internal standards. The internal standards used for determining PAHs and NPAHs were as follows: 6-NC-*d*₁₁ for all of NPAHs, Pyr-*d*₁₀ for Pyr and Flu, BaA-*d*₁₂ for BaA, Chr, BbF, and BkF, and BaP-*d*₁₂ for BaP, DBA, BghiP, and IDP. Calibration curves of each PAHs and NPAHs were prepared by plotting five points (*n*=5) between the lowest concentration of 0.01 - 1 µg/L for PAHs and 0.005 - 5 µg/L for NPAHs, and at the highest concentration of 500 µg/L for PAHs and 10 or 100 µg/L for NPAHs. The LOD and LOQ were determined from lowest concentration at which the signal-to-noise (S/N) ratio was higher than 3 and 10, respectively, with precision less than 15% through the entire treatment of spiked blank samples. The intra-day and inter-day accuracy and precision were examined by repetitive determination of the PM_{2.5} samples spiked with standards at a constant concentration for each analyte, together with non-spiked samples. The accuracy was expressed as the ratio of the quantified concentration to that of the known concentration of the spiked analyte. The precision was calculated as the relative standard deviation (SRD, %) of the replicates. To evaluate the intra-day precision, the spiked samples and non-spiked samples were prepared four times per day. The inter-day precision was determined using independent experiments repeated on five consecutive days. All extracts were analyzed on the same day as the samples were extracted.

2.3 Result and discussion

2.3.1 Column evaluation for the clean-up column

The two-dimensional HPLC system consists of clean-up, reduction, column-switching, separation and FL detection steps (Fig. 2.3). A high percentage of ethanol is necessary for the reduction step using the Pt/Rh column and acetonitrile decreases the reduction efficiency (Hayakawa et al., 2001). On the other hand, acetonitrile is an effective solvent for clear separation of all targets including similar isomers (Tang et al., 2005a) and to decrease column pressure. To eliminate

opposing solvent effects, the two-dimensional system was able to switch solvent source to minimize the solvent effects of the 1st dimension. The clean-up column can be incorporated into the 1st dimension for a partial purification to remove hydrophilic matrix in a sample through the column-switching based on differences in the elution times from the column between the analytes and the sample matrix. Since PAHs and NPAHs have a wide range of hydrophobicities, ODS columns have little effect on removing substances that may interfere with the detection of analytes and require a long time for the elution of all analytes. This is especially true for low abundance NPAHs and consequently, the CL system required laborious pretreatments such as washing sample extracts with sodium hydroxide and sulfuric acid (Tang et al., 2003). Furthermore, our previous CL method required 58 min to trap the analytes on the concentration column and then 108 min to separate only NPAHs (total 166 min) (Tang et al., 2005a). The retention characteristics of 8 reversed-phase columns were evaluated to find a more effective column than the conventional ODS column. The characteristics of the stationary phases are listed in Table 2.1. The ideal characteristics of a clean-up column are a short elution band and large retention factors for all analytes. A shorter band can decrease the loading time to the concentration column and strong retention can increase the specificity of clean-up column. To satisfy these conditions, analytes with low logP values such as DNPs need to be retained with other interactions in addition to hydrophobic interaction, whereas the retention of the analytes with high logP values, such as IDP, needs to be suppressed.

The retention times of PAHs and NPAHs on each column were determined with methanol:water as the mobile phase, because of limitations in the reduction column and incompatibility between acetonitrile and π - π interaction (Snyder et al., 2004). The *k* values of the PAHs and NPAHs are listed in Table 2.4. The hydrophobicity of NPE, PFP and PBr phases is much smaller than that of ODS phases (5C₁₈-MS-II and 5C₁₈-AR-II) and similar to that of the PE phase (Kimata et al., 1992). Nevertheless, mean *k* values of the three phases for NPAHs were comparable to or higher than those of the ODS phases. A non-substituted PAH (Pyr), nitro-substituted PAHs (1-, 2- and 4-NPs) and dinitro-substituted PAHs (1,3-, 1,6- and 1,8-DNPs) were eluted from the 6 tested columns in the following order: Pyr < NPs < DNPs, showing a reversal of the elution order in the ODS columns. The strong retention of NPAHs on NPE and PFP phases compared to the PE phase indicated the presence of strong dipole-dipole interactions (Kimata et al., 1992). In particular, PAHs and NPAHs were strongly retained on PYE and PBr phases, indicating strong dispersive interactions between the aromatic species and the stationary phases in addition to their hydrophobic and π - π interactions (Turowski et al., 2001).

To evaluate the length of the elution band and the distribution of retention times, switching indexes were calculated for each column (Table 2.4). A small value indicates a short elution band and strong retention of the analytes. Although the PE column showed the smallest switching index (0.93), the retention of the analytes on the column was considerably weaker than the other columns. Taking into consideration the separation of the analytes from a hydrophilic matrix, the NPE column (switching index: 1.00) was selected as the clean-up column for the switching column system. Finally, ethanol/acetate buffer (pH 5.5, 95/5, v/v) at a flow rate of 0.2 mL/min was used for the clean-up column as the mobile phase, taking into account the conditions required for the reduction step (Hayakawa et al., 2001). The performance of the NPE column was maintained under the conditions because the switching index showed 1.05, the same value as observed in the conditions for the column evaluation. All the analytes were retained for over 15 min and eluted for 17.6 min (switching time: 15.4 - 33.0 min)

Table 2.4 Capacity factor (k values) of PAHs and NPAHs in the examined columns

Compound	Examined reversed-phase column							
	5C ₁₈ -MS-II	5C ₁₈ -AR-II	PE	π Nap	PYE	NPE	PFP	PBr
PAHs								
Flu	8.27	9.76	2.71	5.97	8.47	4.14	6.73	17.57
Pyr	8.88	10.56	2.83	6.15	8.64	4.44	7.37	18.93
Chr	11.09	13.31	4.33	8.31	12.38	5.65	7.92	20.47
BaA	11.24	13.38	4.62	8.35	11.97	5.74	7.94	21.54
BbF	13.59	16.26	5.48	10.22	15.15	7.58	9.82	21.72
BkF	13.89	16.60	5.59	10.11	15.95	7.80	9.62	26.40
BaP	14.43	17.36	5.66	10.59	16.66	8.28	10.50	28.66
DBA	16.01	19.02	6.90	12.12	17.66	11.20	11.08	29.90
BghiP	16.85	20.46	6.62	12.34	21.20	9.31	11.96	42.62
IDP	16.89	20.44	6.67	12.59	19.93	10.43	12.98	36.10
NPAHs								
9-NA	5.08	6.05	2.61	6.58	11.00	4.62	5.68	13.19
2-NF	4.57	5.78	2.50	7.52	17.32	3.81	4.18	14.91
2-NA	5.82	6.97	3.08	8.41	17.97	4.92	5.42	18.64
9-NPh	5.16	7.43	2.83	7.35	13.83	4.97	5.77	16.19
4-NP	7.82	9.54	4.54	10.34	20.48	7.13	8.46	24.49
3-NFR	7.99	9.59	4.12	10.71	22.86	6.62	7.90	24.15
1-NFR	8.16	10.18	4.03	9.81	19.59	7.22	9.09	24.29
2-NFR	8.14	9.87	4.72	10.84	22.04	7.02	7.87	24.53
1-NP	7.37	9.42	4.04	9.92	21.29	7.07	7.91	24.70
2-NP	8.59	10.52	4.69	11.11	23.15	7.02	8.28	25.83
7-NBaA	9.36	11.18	5.39	11.24	19.23	8.17	9.00	22.43
6-NC	10.08	12.20	5.96	12.99	23.28	8.65	9.28	26.23
1-NPer	9.87	12.20	6.21	13.23	24.03	10.24	8.86	27.22
1,6-DNP	7.73	7.32	5.66	17.25	29.34	9.69	7.48	31.58
1,3-DNP	7.72	9.81	6.87	19.04	30.22	11.11	9.79	31.40
1,8-DNP	6.69	8.64	5.84	17.20	30.38	11.35	10.38	32.05
6NBaP	12.28	15.06	6.94	14.67	27.75	11.54	11.64	32.13
3NPer	12.25	15.32	7.10	16.60	36.97	11.52	10.90	41.11
k max	16.89	20.46	7.10	19.04	36.97	11.54	12.98	42.62
k min	4.57	5.78	2.50	5.97	8.47	3.81	4.18	13.19
k mean	9.85	11.94	4.95	11.13	19.95	7.76	8.71	25.68
k max - k min	12.32	14.69	4.60	13.07	28.50	7.73	8.80	29.42
Switching index	1.25	1.23	0.93	1.17	1.43	1.00	1.01	1.15

2.3.2 Separation and detection of analytes in the 2nd dimension

After column switching, 10 PAHs and 18 amino-derivatives of NPAHs were separated on the separation columns which consist of 2 polymeric-type ODS columns (4.6 mm i.d. \times 250 each, 5 μ m) in tandem. Fig. 2.3 shows typical chromatograms of a standard mixture of target PAHs, NPAHs and deuterated standards, which were all well separated by gradient elution. The reduced NPAHs were eluted from the columns faster than non-substituted PAHs. Three deuterated PAHs (Pyr- d_{10} , BaA- d_{12} , BaP- d_{12}) and the amino-derivative of 6-NC- d_{11} were separated from the non-deuterated compounds with sufficient resolution ($R_s > 2.85$). In general, stable isotope-labeled compounds are excellent internal standards for mass spectrometric detection, but not for optical detection methods such as FL detection. However, deuterated PAHs can be separated from the non-deuterated analogues with baseline resolution on polymeric-type ODS columns and have nearly the same fluorescence characteristics (Toriba et al., 2003). Furthermore, we have successfully applied deuterated PAHs, 1-NP and hydroxylated PAHs to HPLC-FL methods for environmental and biological samples (Toriba et al., 2007; Ohno et al., 2009). Total analytical time for simultaneously determining PAHs and NPAHs was 120.0 min including 33.0 min for clean-up and reduction steps in the 1st dimension. After the 1st dimension, the 2nd dimension required 52.0 min and 82.0 min to separate NPAHs (elution time: 45.0 - 85.0 min) and PAHs (elution time: 79.0 - 115.0 min), respectively. The analysis time for NPAHs was reduced by half compared to the HPLC-CL method (Tang et al., 2005a) and was followed by PAH analysis.

Moreover, we focused on the separation of nitrofluoranthrenes and nitropyrenes which display similar chromatographic behavior on reversed-phase columns. Specifically, 2-NFR and 2-NP are well-known secondary products formed via atmospheric reactions of their parent PAHs and have been frequently found in PM samples (Hayakawa et al., 2011; Tang et al., 2014). It is essential to separate these two compounds from the other detectable isomers (1-NFR, 3-NFR, 1-NP and 4-NP) in real samples. The 6 compounds were successfully separated on the 2 ODS columns in tandem which required a high number of theoretical plates (Fig. 2.3). Dual-channel fluorescence detection was used to avoid frequent changes of the detection wavelengths or separate co-eluted or closely eluting peaks. The two-channel program was set based on optimal excitation and emission wavelengths of the analytes (Cvačka et al., 1998; Šoustek et al., 2008). Remarkably, 6-NC and 7-NBaA were successfully separated by their specific excitation and emission wavelengths, not by the separation columns as they had identical retention times (Fig. 2.3). The signal arising from one analyte was not detected on the other channel. These results suggest that the proposed HPLC-FL system is suitable for the identification of all target compounds.

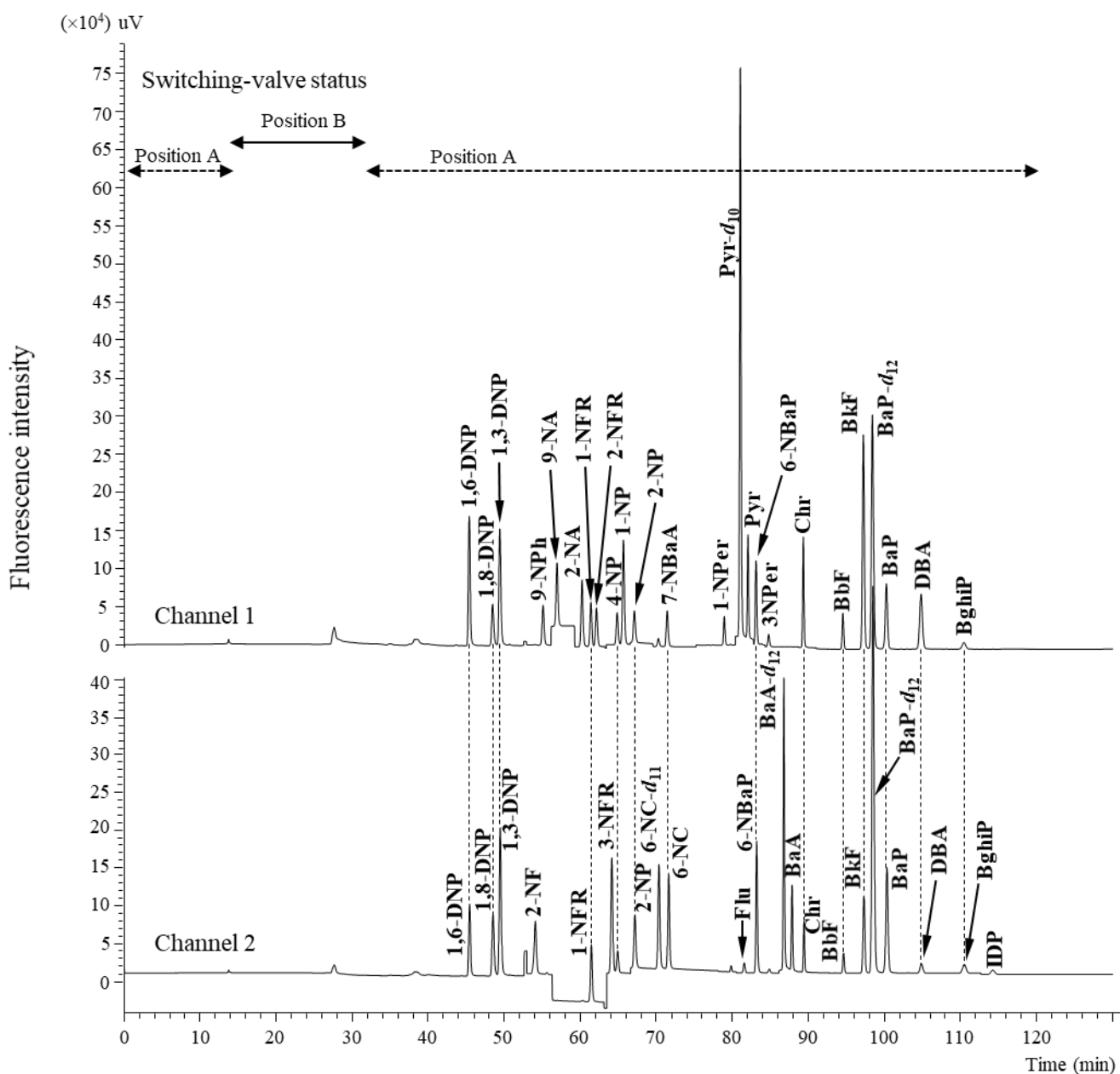


Fig.2.3. Representative standard chromatograms of PAHs and NPAHs measured by the developed HPLC-FL method

Injected amounts: Channel 1; 1,6-DNP, 750 pg; 9-NPh, 3 ng; 9-NA, 3 ng; 2-NA, 500 pg; 2-NFR, 1 ng; 4-NP, 12.5; 1-NP, 500 pg; 7-NBaA, 1 ng; 1-Nper, 10 ng; Pry-d10, 11 ng; Pyr, 1 ng; 3NPer, 10 ng; Chr, 1 ng; BbF, 1 ng; BkF, 1 ng; DBA, 1 ng; Channel 2; 1,8-DNP, 1 ng; 1,3-DNP, 1 ng; 2-NF, 500 pg; 1-NFR, 1 ng; 3-NFR, 5 ng; 2-NP, 4 ng; 6-NC-d11 6 ng; 6-NC, 1 ng; Flu, 1 ng; ; 6-NBaP, 6 ng; BaA-d12, 6 ng; BaA, 1 ng; BaP-d12, 7 ng; BaP, 1 ng; BghiP, 1 ng; IDP, 1 ng.

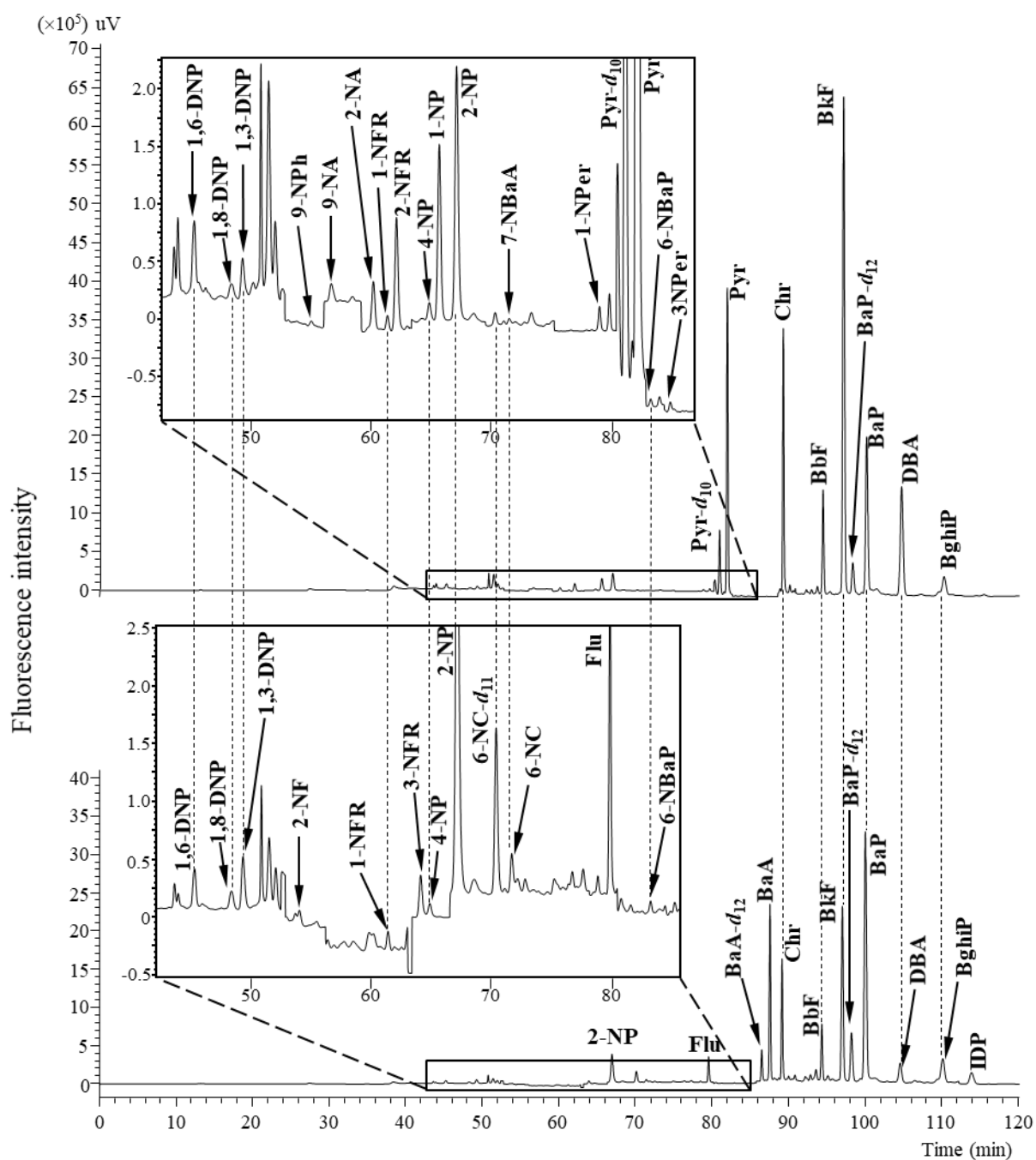


Fig.2.4. Representative chromatograms resulting from a crude extract of the spiked PM_{2.5} samples.

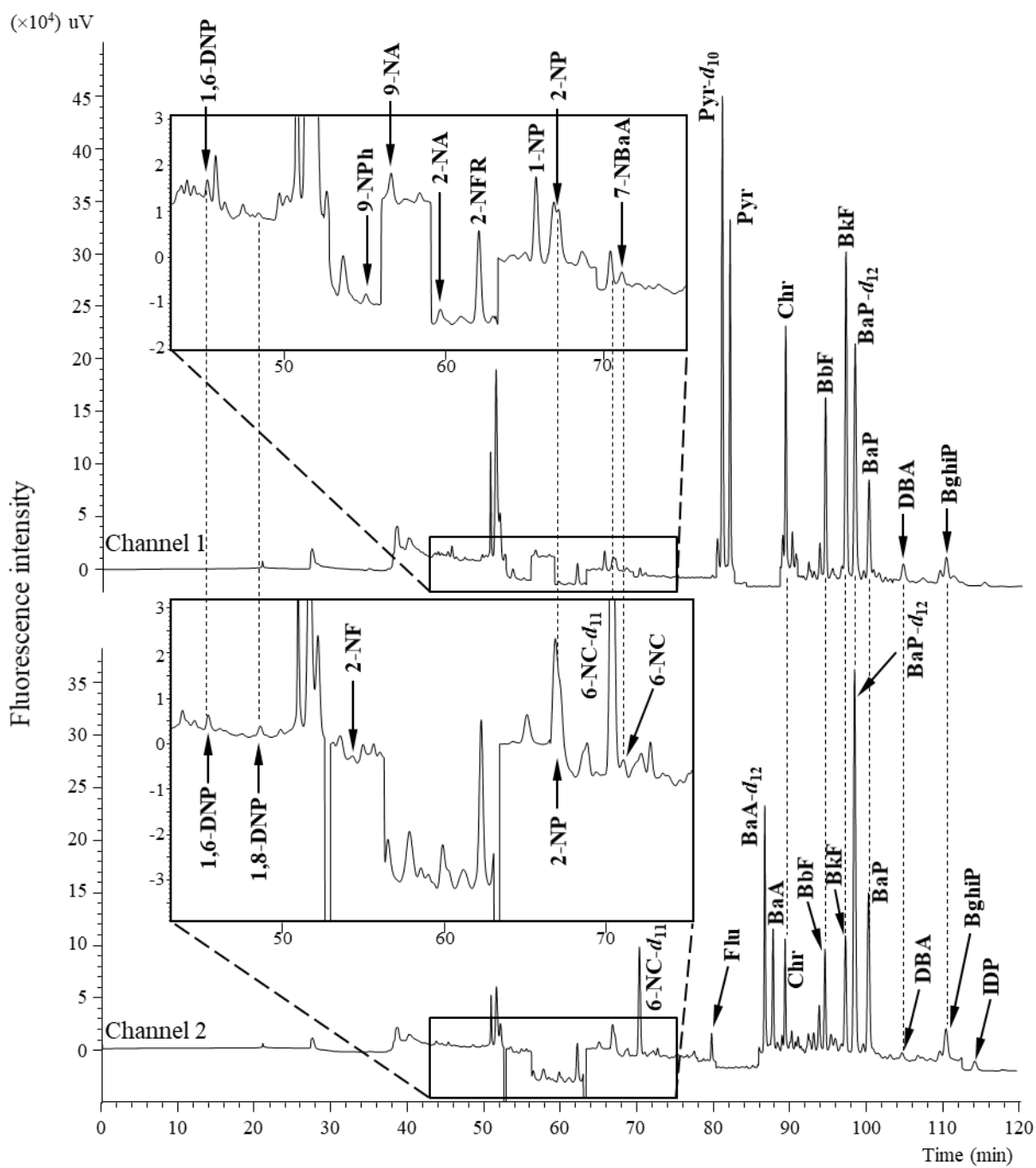


Fig.2.5. Representative chromatograms resulting from the crude extract of SRM 1649b.

2.3.3 Limit of detection (LOD), limit of quantitation (LOQ) and calibration curve

The concentrations of the analytes which establish S/N ratio of 3:1 and 10:1 were considered to be the LODs and LOQs, respectively. The LODs and LOQs for PAHs and NPAHs are listed in Table 2.5, indicating that the developed method is sensitive enough to measure the analytes in the PM samples (1.1 pg/m³ for 3-NPer which showed the lowest sensitivity). Compared to previous HPLC-CL method (Tang et al., 2005a), the LODs of 2-NF, 9-NPhe, 2-NA, 1-NP, 2-NP 7-NBaA and 6-NC were substantially improved, whereas the sensitivities of 9-NA, 1- and 3-NPer were decreased. The sensitivities of the PAHs were higher than those previously reported because of instrumental improvement (Toriba et al., 2003). Calibration curves were constructed from the peak area ratio of the analyte to deuterated internal standards and good linearity ($r^2 > 0.9999$ for PAHs and > 0.99 for NPAHs) was observed for all calibration curves, as shown in Table 2.5.

2.3.4 Accuracy and precision

The extraction method of PAHs and NPAHs in PM samples involved ultrasonic extraction with DCM, evaporation and redissolving steps, and then the crude extract was directly injected to the HPLC system. The analysis of the crude extracts is difficult to perform with GC-MS. The recoveries of the deuterated internal standards (Pyr-*d*₁₀, BaA-*d*₁₂, BaP-*d*₁₂ and 6-NC-*d*₁₁) were 80 - 119% through the entire pretreatment. Known amounts of PAHs and NPAHs were added to PM_{2.5} samples, and their quantification accuracy and precision were evaluated (Table 2.6 and 2.7). The accuracies were 86 - 98% and 87 - 109% for the PAHs and NPAHs, respectively. The precision was good with a RSD of 15% or less for all analytes. Both intra- and inter-day accuracy and precision were satisfactory for the simultaneous determination of all PAHs and NPAHs in the PM_{2.5} samples. Fig. 2.4 shows typical chromatograms resulting from a crude extract of the spiked PM_{2.5} samples. The sample matrix did not interfere with the identification and quantification of the analytes in the chromatograms. The results suggest effective fractionation of the analytes from the sample matrix by the clean-up column and column-switching in the 1st dimension.

Table 2.5 Limits of detection (LOD), limits of quantification (LOQ) and calibration curves of PAHs and NPAHs by the proposed HPLC-FL method.

Compound	LOD ^a (pg/injection)	LOQ ^b (ng/L)	Calibration Range (µg/L)	Linearity (r ²)	LOD ^c (HPLC-CL) (pg/injection)
PAHs					
Flu	4.0	130	1-500	0.9999	-
Pyr	0.4	12	0.05-500	1.0000	-
BaA	0.4	13	0.05-500	1.0000	-
Chr	0.6	18	0.05-500	1.0000	-
BbF	0.7	24	0.05-500	1.0000	-
BkF	0.1	4.3	0.01-500	1.0000	-
BaP	0.3	8.7	0.01-500	1.0000	-
DBA	0.4	14	0.05-500	1.0000	-
BghiP	5.0	170	1-500	1.0000	-
IDP	9.2	310	1-500	0.9999	-
NPAHs					
1,6-DNP	0.4	12	0.05-10	0.9960	0.3
1,8-DNP	1.1	37	0.05-10	0.9957	0.3
1,3-DNP	0.5	15	0.05-10	0.9947	0.3
2-NF	0.5	17	0.05-10	0.9959	210
9-NA	9.3	310	0.5-10	0.9942	0.9
9-NPh	2.6	87	0.1-10	0.9983	27
2-NA	0.1	2.2	0.005-10	0.9928	2.2
1-NFR	1.6	52	0.1-10	0.9948	-
2-NFR	0.7	23	0.05-10	0.9957	-
3-NFR	25	820	1-100	0.9953	5.2
4-NP	27	890	1-100	0.9974	7.4
1-NP	0.1	3.1	0.005-10	0.9960	2.5
2-NP	1.9	64	0.1-10	0.9954	37
7-NBaA	1.0	34	0.05-10	0.9978	2.7
6-NC	0.5	17	0.05-10	0.9951	41
1-NPer	87	2900	5-100	0.9976	1.2
6-NBaP	8.9	300	0.5-10	0.9970	0.6
3-NPer	140	4500	5-100	0.9946	0.6

^a Limit of detection (S/N = 3), ^b Limit of quantification of the entire method (precision <25%, S/N = 10), ^c Tang et al., 2005

Table 2.6 Precision and accuracy in the analyzed results of PAHs (n=4)

Compound	Spiked conc. (pg/m ³)	Observed (mean \pm SD, pg/m ³) ^a						Accuracy (%) ^b		Precision (RSD%)	
		Intra-day			Inter-day			Intra-day	Inter-day	Intra-day	Inter-day
Flu	0	133	\pm	4.8	129	\pm	6.3	98	96	3.6	4.9
	140	269	\pm	9.1	264	\pm	7.3			3.4	2.8
Pyr	0	93	\pm	10	86	\pm	4.4	97	99	11	5.1
	140	229	\pm	9.1	224	\pm	7.9			4.0	3.5
BaA	0	25	\pm	0.7	25	\pm	1.4	98	94	2.8	5.9
	140	162	\pm	6.4	157	\pm	7.6			4.0	4.9
Chr	0	50	\pm	2.4	49	\pm	4.3	98	95	4.9	8.9
	140	186	\pm	6.1	181	\pm	10.2			3.3	5.6
BbF	0	50	\pm	5.3	54	\pm	9.6	89	90	10	18
	140	175	\pm	5.3	181	\pm	16.3			3.1	9.0
BkF	0	20	\pm	1.9	19	\pm	2.1	93	89	9.4	11
	140	151	\pm	9.7	144	\pm	15.4			6.4	11
BaP	0	31	\pm	0.1	30	\pm	3.0	90	88	0.3	10
	140	156	\pm	4.8	152	\pm	6.7			3.1	4.4
DBA	0	6.5	\pm	0.6	7.0	\pm	0.5	90	89	9.0	6.9
	140	132	\pm	3.5	131	\pm	4.5			2.6	3.5
BghiP	0	41	\pm	1.6	40	\pm	4.7	87	86	4.0	12
	140	163	\pm	7.3	161	\pm	4.4			4.5	2.8
IDP	0	44	\pm	2.7	41	\pm	4.5	96	90	6.1	11
	140	178	\pm	3.3	167	\pm	8.4			1.8	5.0

^a The recovered concentrations were calculated as the difference between the concentrations of the spiked samples and mean concentration of non-spiked samples.

^b Accuracy is expressed as the percentage of accuracy [(mean observed concentration/ spiked concentration) \times 100].

Table 2.7 Precision and accuracy in the analyzed results of NPAHs (n=4)

Compound	Spiked conc. (pg/m ³)	Observed (mean \pm SD, pg/m ³) ^a						Accuracy (%) ^b		Precision (RSD%)	
		Intra-day			Inter-day			Intra-day	Inter-day	Intra-day	Inter-day
1,6-DNP	0 1.39	0.03 \pm 0.00 1.36 \pm 0.11	0.03 \pm 0.00 1.40 \pm 0.18					96	99	7.8 8.3	6.6 13
1,8-DNP	0 1.39	0.16 \pm 0.01 1.49 \pm 0.14	0.15 \pm 0.01 1.49 \pm 0.16					96	96	3.5 9.4	4.1 11
1,3-DNP	0 1.39	- ^c 1.49 \pm 0.07	- 1.47 \pm 0.09					107	106	4.5	6.2
2-NF	0 1.39	< LOQ ^d 1.52 \pm 0.01	< LOQ 1.46 \pm 0.11					109	105	0.7	7.6
9-NPh	0 2.78	0.28 \pm 0.02 2.95 \pm 0.15	0.26 \pm 0.04 2.75 \pm 0.14					96	90	8.2 5.2	14 5.1
9-NA	0 2.78	5.13 \pm 0.18 7.68 \pm 0.33	4.93 \pm 0.14 7.80 \pm 0.36					92	103	3.5 4.3	2.8 4.7
2-NA	0 1.39	- 1.42 \pm 0.07	- 1.44 \pm 0.12					102	104	5.2	8.3
1-NFR	0 1.39	0.01 \pm 0.00 1.22 \pm 0.05	0.01 \pm 0.00 1.26 \pm 0.11					87	90	7.8 4.0	7.0 8.3
2-NFR	0 11.1	1.67 \pm 0.14 12.5 \pm 0.71	1.68 \pm 0.15 13.2 \pm 1.24					97	104	8.6 5.7	9.0 9.4
3-NFR	0 6.94	0.84 \pm 0.12 7.33 \pm 0.14	0.85 \pm 0.13 7.21 \pm 0.37					93	92	14 1.9	15 5.1
4-NP	0 2.78	0.09 \pm 0.00 2.77 \pm 0.11	0.08 \pm 0.01 2.66 \pm 0.18					96	93	4.4 3.9	13 6.6
1-NP	0 2.78	0.45 \pm 0.01 3.14 \pm 0.24	0.46 \pm 0.02 3.06 \pm 0.24					97	93	2.2 7.6	3.8 7.8
2-NP	0 12.5	0.76 \pm 0.03 12.6 \pm 0.46	0.79 \pm 0.04 12.4 \pm 0.74					95	93	4.5 3.6	5.6 5.9
7-NBaA	0 1.39	- 1.37 \pm 0.03	- 1.34 \pm 0.06					99	96	2.1	4.2
6-NC	0 0.28	- 0.26 \pm 0.02	- 0.28 \pm 0.04					92	101	6.2	14
1-NPer	0 43.1	- 39.5 \pm 3.19	- 38.4 \pm 2.31					92	89	8.1	6.0
6-NBaP	0 2.78	- 2.66 \pm 0.13	- 2.62 \pm 0.18					96	94	4.9	7.0
3-NPer	0 43.1	- 37.4 \pm 1.61	- 37.6 \pm 1.43					87	87	4.3	3.8

^a The recovered concentrations were calculated as the difference between the concentrations of the spiked samples and mean concentration of non-spiked samples.

^b Accuracy is expressed as the percentage of accuracy [(mean observed concentration/ spiked concentration) \times 100].

2.3.5 Quantitative determination of atmospheric samples

The concentrations of PAHs in the suburban PM_{2.5} samples used for the validation study (the concentrations of non-spiked samples in Table 2.6) were slightly higher than those in a background site located in the Noto peninsula without any major sources near the station (Tang et al., 2014). In contrast, the PAH levels were lower than samples collected at a road side in Kanazawa city (Tang et al., 2005b) and the other Japanese cities (Naser et al., 2008; Hayakawa et al., 2016b). PAHs are commonly found in PM_{2.5} fractions and PAH concentrations have been directly correlated with PM_{2.5} levels (Naser et al., 2008). The PAH concentrations in this study were reasonable, with respect to the PM_{2.5} levels of $7.7 \pm 2.9 \mu\text{g}/\text{m}^3$ measured during the sampling period. Among the NPAHs, 1,3-DNP, 2-NA and NPAHs having higher molecular weight were not detected in the PM_{2.5} samples (Table 2.7). The concentration of 1-NP in this study was significantly lower than other sites, including the background site (Naser et al., 2008; Tang et al., 2014; Hayakawa et al., 2016b), indicating little emission source related to automobile exhaust around the site. The 1-NP contribution to NPAHs has substantially decreased throughout Japan in recent years, in conjunction with government regulations regarding automobile emissions (Kojima et al., 2010).

Using the developed HPLC-FL method, we also quantified PAHs and NPAHs in an urban dust standard reference material (SRM1649b). Fig. 2.5 shows representative chromatograms resulting from the crude extract of SRM 1649b. The obtained concentrations of 10 PAHs and 18 NPAHs were compared with certified concentrations by NIST and data reported from other studies (Table 2.8). The quantified PAH concentrations showed good agreement with the certified values in the range of 90 - 105% (NIST 2016). Five NPAHs were highly consistent with the certified NPAH values (92 - 117%), except for 9-NA, 9-NPh and 3-NFR. Several compounds showed similar concentrations with other literature data (Schantz et al., 2012; Albinet et al., 2014). There were large variations in the reported concentrations of NPAHs from literature data (Table 6), and these differences could arise from all of the procedure of the analytical protocols employed. The NPAH concentrations determined by pressurized solvent extraction (PLE) reported by Schantz et al. (2012) were very close to those in the certificate. On the other hand, Albinet et al. (2014) used two extraction methods, PLE and the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) approaches for the NPAHs in SRM1649b. They reported concentrations of numerous NPAHs which included analytes not reported in the certificate with several other being different from the certificate data and having variations between the extraction methods. The variation of the quantified data may be from the difference among general Soxhlet extraction, PLE and sonication.

Table 2.8 Comparison of quantified concentrations of PAHs (mg/kg) and NPAHs(µg/kg) in SRM1649b in this study to the literatures

	This study	NIST Certificate	Schantz et al.	Albinet et al.	Albinet et al.
		(2016)	(2012)	(2014)	(2014)
Number of replicates	n = 3	n = 3	n = 3	n = 6	n = 6
Analytical instrument	HPLC-FL	GC-NICIMS	GC-NICIMS	GC-NICIMS (PLE)	GC-NICIMS (QuEChERS)
PAHs					
Flu	6.1	6.2	6.5	- ^a	-
Pyr	5.2	5.0	4.9	-	-
Chr	2.1	2.1	2.9	-	-
BaA	3.0	3.0	2.2	-	-
BbF	5.7	6.2	7.7	-	-
BkF	1.7	1.7	1.8	-	-
BaP	2.5	2.5	2.9	-	-
DBA	0.3	0.3	0.5	-	-
BghiP	3.6	4.0	-	-	-
IDP	2.6	2.9	2.6	-	-
NPAHs					
1,6-DNP	12.1	-	-	9.8	45.3
1,8-DNP	23.1	-	-	29.4	61.8
1,3-DNP	<LOQ ^c	-	-	nd ^b	nd
2-NF	20.1	-	-	nd	53.0
9-NA	126	243	255.0	36.1	25.7
9-NPh	44.8	1.8	1.7	11.4	15.3
2-NA	25.8	-	-	33.4	55.7
1-NFR	<LOQ	-	-	-	-
2-NFR	279	304	307.7	322.8	261.9
3-NFR	<LOQ	4.7	4.1	nd	nd
4-NP	<LOQ	-	-	13.7	27.3
1-NP	72.0	74.8	73.2	104.1	60.9
2-NP	12.7	10.9	11.1	41.0	42.0
7-NBaA	23.6	24.4	24.0	69.3	35.2
6-NC	3.9	3.6	3.3	11.6	18.8
1-NPer	<LOQ	-	-	-	-
6-NBaP	<LOQ	-	-	79.9	66.6
3-NPer	<LOQ	-	-	-	-

^a No data, ^b Not detected, ^c less than limit of detection

2.4 Conclusions

A two-dimensional HPLC-FL system has been developed for the simultaneous determination of 10 PAHs and 18 NPAHs. The number of the analytes was increased and a flow pass of the 2nd dimension simplified the whole system compared to the previous simultaneous analysis (Murahashi et al., 1994). The single analysis can cover particle-bound PAHs and NPAHs required for the evaluations of strong mutagenic compounds, combustion sources and atmospheric reactions. The column switching system enables direct injection of crude PM sample extract without complicated pretreatment. The simple pretreatment can shorten the total analytical time and increase recoveries of the analytes. The NPE column as the clean-up column showed short elution band and strong retention of the analytes by dipole-dipole interactions and displayed effective fractionation of the analytes from the sample matrix in the 1st dimension. Good separation of all target compounds was achieved by polymeric-type ODS columns and dual-channel FL detection in the second dimension. The time required for analysis of NPAHs was cut in half when compared to conventional HPLC-CL methods with a total time of 120 min, including analysis of PAHs. The LODs of PAHs and NPAHs were better than or comparable to those of the previous FL methods for PAHs and the CL methods for NPAHs. The FL detection can reduce solvent consumption and eliminate the requirement for pumps for the post-label reagents for the CL detection of NPAHs. This HPLC-based method also enables much larger-scale injections (100 μ L or more) compared to GC-based methods, therefore, it may be applicable for samples with a small sampling volume limited by collecting instruments such as personal samplers. The quantification of PAHs and NPAHs in crude extracts of PM_{2.5} samples showed good accuracy and precision and concentration determinations for SRM 1649b were similar to the certificate and other values reported in the literature. Considering the above-mentioned advantages, the developed method has the potential to a standard HPLC-based method, especially for NPAH determination.

References

- Ahmed, T. M., Bergvall, C., Åberg, M., Westerholm, R. (2015) Determination of oxygenated and native polycyclic aromatic hydrocarbons in urban dust and diesel particulate matter standard reference materials using pressurized liquid extraction and LC-GC/MS. *Analytical and Bioanalytical Chemistry*, 407, 427–438.
- Albinet, A., Leoz-Garziandia, E., Budzinski, H., Villenave, E. (2006) Simultaneous analysis of oxygenated and nitrated polycyclic aromatic hydrocarbons on standard reference material 1649a (urban dust) and on natural ambient air samples by gas chromatography–mass spectrometry with negative ion chemical ionisation. *Journal of Chromatography A*, 1121, 106–113.
- Albinet, A., Leoz -Garziandia, E., Budzinski, H., Villenave, E. (2006) Simultaneous analysis of oxygenated and nitrated polycyclic aromatic hydrocarbons on standard reference material 1649a (urban dust) and on natural ambient air samples by gas chromatography–mass spectrometry with negative ion chemical ionisation. *Journal of Chromatography A*, 1121, 106–113.
- Albinet, A., Leoz-Garziandia, E., Budzinski, H., & Villenave, E. (2007) Sampling precautions for the measurement of nitrated polycyclic aromatic hydrocarbons in ambient air. *Atmospheric Environment*, 41, 4988-4994.
- Albinet, A., Nalin, F., Tomaz, S., Beaumont, J., Lestremau, F. (2014) A simple QuEChERS like extraction approach for molecular chemical characterization of organic aerosols: Application to nitrated and oxygenated PAH derivatives (NPAH and OPAH) quantified by GC-NICIMS. *Analytical and Bioanalytical Chemistry*, 406, 3131–3148.
- Abdel-Shafy, H. I., & Mansour, M. S. (2016) A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25(1), 107-123.
- Alves, C. A., Vicente, A. M. P., Gomes, J., Nunes, T., Duarte, M., Bandowe, B. A. M. (2016) Polycyclic aromatic hydrocarbons (PAHs) and their derivatives (oxygenated -PAHs, nitrated -PAHs and azaarenes) in size-fractionated particles emitted in an urban road tunnel. *Atmospheric Research*, 180, 128-137.
- Ames, B. N., McCann, J., Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Research/Environmental Mutagenesis and Related Subjects*, 31(6), 347-363.
- Andrade-Eiroa, A., Leroy, V., Dagaut, P., (2010) Advances in PAH/nitro-PAH fractioning. *Anal. Methods* 2, 2014–2017.
- Andreou, G., Rapsomanikis, S. (2009) Polycyclic aromatic hydrocarbons and their oxygenated derivatives in the urban atmosphere of Athens. *Journal of Hazardous Materials*, 172, 363–373.
- Araki, Y., Tang, N., Ohno, M., Kameda, T., Toriba, A., Hayakawa, K. (2009) Analysis of atmospheric polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in gas/particulate phases separately collected by a high -volume air sampler equipped with a column packed with XAD-4 resin. *Journal of Health Science*, 55, 77-85.
- Arey, J., Zielinska, B., Atkinson, R., Winer, A. M., Ramdahl, T., Pitts, J. N. (1986) The formation of nitro -PAH from the gas-phase reactions of fluoranthene and pyrene with the OH radical in the presence of NO_x. *Atmospheric Environment*, 20, 2339-2345.

- Atkinson, R. Arey, J. (1994). Atmospheric Chemistry of Gas-phase polycyclic Aromatic Hydrocarbons: Formation of Atmospheric Mutagens. *Environmental Health Perspective*. 102:117-126.
- ATSDR (Agency for Toxic Substance and Disease Registry). 1995 Toxicological profile for polycyclic aromatic hydrocarbons (PAHs). Available at <http://www.atsdr.cdc.gov/toxprofiles/tp69.html>.
- Basheer, C., Balasubramanian, R., Lee, H. K. (2003) Determination of organic micropollutants in rainwater using hollow fiber membrane/liquid-phase microextraction combined with gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1016(1), 11-20.
- Bamford, H. A., Baker, J. E. (2003) Nitro-polycyclic aromatic hydrocarbon concentrations and sources in urban and suburban atmospheres of the Mid-Atlantic region. *Atmospheric Environment*, 37(15), 2077-2091.
- Bandowe, B. A. M., Meusel, H., Huang, R., Ho, K., Cao, J., Hoffmann, T., Wilcke, W. (2014) PM_{2.5}-bound oxygenated PAHs, nitro-PAHs and parent-PAHs from the atmosphere of a Chinese megacity: Seasonal variation, sources and cancer risk assessment. *Science of the Total Environment*, 473–474, 77–87.
- Bandowe, B. A. M., Meusel, H. (2014) PM_{2.5}-bound PAHs, nitro-PAHs and parent-PAHs from the atmosphere of a Chinese megacity: Seasonal variation, sources and cancer risk assessment. *Science of The Total Environment*, 473-474, 77-87.
- Bandowe, B. A. M., Meusel, H. (2017) Nitrated polycyclic aromatic hydrocarbons (nitro PAHs) in the environment—A review. *Science of The Total Environment*, 581-582, 237-257.
- Bin. L. L., Yan, L., Lin, J. M., Ning, T., Hayakawa, K., Mameda, T. (2007) Development of analytical methods for polycyclic aromatic hydrocarbons (PAHs) in airborne particulates: a review. *Journal of Environmental Sciences*, 19(1), 1-11.
- Brorström-Lundén, E., Remberger, M., Kaj, L., Hansson, K., Palm Cousins, A., Andersson, H., Haglund, P., Ghebremeskel, M., Schlabach, M., (2010) Results From the Swedish National Screening Programme 2008: Screening of Unintentionally Produced Organic Contaminants. Swedish Environmental Research Institute (IVL) report B1944, Göteborg, Sweden. <http://www.ivl.se/download/18.7df4c4e812d2da6a416800072123/B1944.pdf>
- Chang, K. F., Fang, G. C., Chen, J. C., Wu, Y. S. (2006) Atmospheric polycyclic aromatic hydrocarbons (PAHs) in Asia: a review from 1999 to 2004. *Environmental Pollution*, 142(3), 388-396.
- Chen, Y. J., Feng, Y. L., Xiong, S. C., Liu, D. Y., Wang, G., Sheng, G. Y., Fu, J. M. (2011) Polycyclic aromatic hydrocarbons in the atmosphere of Shanghai, China. *Environmental Monitoring and Assessment*, 172, 235-247.
- Cheng, J. P., Yuan, T., Wu, Q., Zhao, W. C., Xie, H. Y., Ma, Y. G., Wang, W. H. (2007) PM₁₀ - bound polycyclic aromatic hydrocarbons (PAHs) and cancer risk estimation in the atmosphere surrounding an industrial area of Shanghai, China 2007. *Water, Air, and Soil Pollution*, 183, 437-446.
- Cheng, Z., Jiang, J., Fajardo, O., Wang, S., Hao, J. (2013) Characteristics and health impacts of particulate matter pollution in China (2001–2011). *Atmospheric Environment*, 65, 186-194.
- Cheruiyot, N.K., Lee, W.-J., Mwangi, J.K., Wang, L.-C., Lin, N.-H., Lin, Y.-C., Cao, J., Zhang, R., Chang-Chien, G.-P. (2015) An Overview: Polycyclic aromatic hydrocarbon emissions from the stationary and mobile sources and in the ambient air. *Aerosol and Air Quality Research*, 15, 2730-2762.
- Choi H, Harrison R, Komulainen H. (2010) Polycyclic aromatic hydrocarbons. In: WHO Guidelines for Indoor Air Quality: Selected Pollutants. Geneva: World Health Organization.

- Chuesaard, T., Chetiyakornkul, T., Kameda, T., Hayakawa, K., Toriba, A. (2014) Influence of biomass burning on the levels of atmospheric polycyclic aromatic hydrocarbons and their nitro derivatives in Chiang Mai, Thailand. *Aerosol Air Quality Research*, 14, 1247-1257.
- Ciccioli, P., Cecinato, A., Brancaleoni, E., Frattoni, M., Zacchei, P., Miguel, A. H., Vasconcellos, P. C. (1996) Formation and transport of 2-nitrofluoranthene and 2-nitropyrene of photochemical origin in the troposphere. *Journal of Geophysical Research*, 101, 19567-19581
- Cochran, R. E., Dongari, N., Jeong, H., Beránek, J., Haddadi, S., Shipp, J., Kubátová, A. (2012) Determination of polycyclic aromatic hydrocarbons and their oxy -, nitro -, and hydroxy - oxidation products. *Analytica Chimica Acta*, 740, 93–103.
- Collins, J., Brown, J., Alexeeff, G., Salmon, A. (1998) Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. *Regulatory. Toxicology. Pharmacology*. 28, 45–54.
- Crimmins, B. S., Baker, J. E. (2006) Improved GC/MS methods for measuring hourly PAH and nitro-PAH concentrations in urban particulate matter. *Atmospheric Environment*, 40(35), 6764-6779.
- Cvačka, J., Barek, J., Zima, J., Fogg, A. G., Moreira, J. C. (1998) Critical Review. High-performance liquid chromatography of nitrated polycyclic aromatic hydrocarbons. *Analyst*, 123(2), 9R-18R.
- Delgado-Saborit, J. M., Stark, C., Harrison, R. M. (2013) Use of a versatile high efficiency multiparallel denuder for the sampling of PAHs in ambient air: gas and particle phase concentrations, particle size distribution and artifact formation. *Environmental Science & Technology*, 48(1), 499-507.
- Delhomme, O., Millet, M., Herckes, P. (2008) Determination of oxygenated polycyclic aromatic hydrocarbons in atmospheric aerosol samples by liquid chromatography–tandem mass spectrometry. *Talanta*, 74, 703–710.
- Dimashki, M., Harrad, S., Harrison, R.M. (2000) Measurement of Nitro-PAHs in the Atmosphere of two cities. *Atmospheric Environment*, 34, 2459-2469.
- Dimashki, M., Lim, L. H., Harrison, R. M., Harrad, S. (2001) Temporal trends, temperature dependence, and relative reactivity of atmospheric polycyclic aromatic hydrocarbons. *Environmental science & technology*, 35(11), 2264-2267.
- Ding, J., Zhong, J., Yang, Y., Li, B., Shen, G., Su, Y., Wang, R. (2012) Occurrence and exposure to polycyclic aromatic hydrocarbons and their derivatives in a rural Chinese home through biomass fuelled cooking. *Environmental Pollution*, 169, 160-166.
- Durant, J.L., Busby, W.F., Lafleur, A.L., Penman, B.W., Crespi, C.L. (1996) Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. *Mutation. Research/Genetic. Toxicology*, 371, 123–157.
- Englert, N. (2004) Fine particles and human health—a review of epidemiological studies. *Toxicology Letters*, 149(1), 235-242.
- EU Air Quality Objectives: <http://ec.europa.eu/environment/air/quality/standards.htm>.
- Garban, B., Blanchoud, H., Motelay-Massei, A., Chevreuil, M., Ollivon, D. (2002) Atmospheric bulk deposition of PAHs onto France: trends from urban to remote sites. *Atmospheric Environment*, 36(34), 5395-5403.
- Garcia, K. O., Teixeira, E. C., Agudelo-Castañeda, D. M., Braga, M., Alabarse, P. G., Wiegand, F., Silva, L. F. (2014) Assessment of nitro-polycyclic aromatic hydrocarbons in PM 1 near an area of heavy-duty traffic. *Science of the Total Environment*, 479, 57-65.

- Golomb, D., Barry, E., Fisher, G., Varanusupakul, P., Koleda, M., Rooney, T. (2001) Atmospheric deposition of polycyclic aromatic hydrocarbons near New England coastal waters. *Atmospheric Environment*, 35(36), 6245-6258.
- Grosse, S., Letzel, T. (2007) Liquid chromatography/atmospheric pressure ionization mass spectrometry with post-column liquid mixing for the efficient determination of partially oxidized polycyclic aromatic hydrocarbons. *Journal of Chromatography A*, 1139, 75–83.
- Grudzinski, J. (2007) Determination of Major Particulate Matter Components (PM₁₀) in Urban Aerosol from Taiyuan City, China (Master's thesis).
- Hasei, T., Watanabe, T., Hirayama, T., (2006) Determination of 3,6-dinitrobenzo[e]pyrene in surface soil and airborne particles by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A*, 1135, 65–70.
- Halsall, C. J., Sweetman, A. J., Barrie, L. A., Jones, K. C. (2001) Modelling the behaviour of PAHs during atmospheric transport from the UK to the Arctic. *Atmospheric Environment*, 35(2), 255-267.
- Hayakawa, K., Murahashi, T., Butoh, M., & Miyazaki, M. (1995) Determination of 1,3-, 1,6-, and 1,8-dinitropyrenes and 1-nitropyrene in urban air by high-performance liquid chromatography using chemiluminescence detection. *Environmental Science and Technology*, 29, 928–932.
- Hayakawa, K., Kawaguchi, Y., Murahashi, T., Miyazaki, M. (1995) Distribution of nitropyrenes and mutagenicity in airborne particulates collected with an Andersen sampler. *Mutation Research Letters*, 348(2), 57-61.
- Hayakawa, K., Terai, N., Dinning, P. G., Akutsu, K., Iwamoto, Y., Etoh, R., Murahashi, T. (1996) An On-line Reduction HPLC/Chemiluminescence Detection System for Nitropolycyclic Aromatic Hydrocarbons and Metabolites. *Biomedical Chromatography*, 10(6), 346-350.
- Hayakawa, K., Murahashi, T., Akutsu, K., Kanda, T., Tang, N., Kakimoto, H., Kizu, R. (2000) Comparison of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in airborne and automobile exhaust particulates. *Polycyclic Aromatic Compounds*, 20, 179.
- Hayakawa, K., Noji, K., Tang, N., Toriba, A., Kizu, R. (2001) A high-performance liquid chromatographic system equipped with on-line reducer, clean-up and concentrator columns for determination of trace levels of nitropolycyclic aromatic hydrocarbons in airborne particulates. *Analytica Chimica Acta*, 445, 205-212.
- Hayakawa, K., Tang, N., Sato, K., Izaki, A., Tatematsu, M., Hama, H., Li Y., Kameda T., Toriba, A. (2011a) Development of HPLC determination method for trace levels of 1-, 2-nitropyrenes and 2-nitrofluoranthene in airborne particulates and its application to samples collected at Noto Peninsula. *Asian Journal of Atmospheric Environment*, 5(3), 146-151.
- Hayakawa, K., Bekki, K., Yoshita, M., Tachikawa, C., Kameda, T., Tang, N., Hosoi, S. (2011b) Estrogenic/antiestrogenic activities of quinoid polycyclic aromatic hydrocarbons. *Journal of Health Science*, 57, 274.
- Hayakawa, K. (2016). Environmental behaviors and toxicities of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons. *Chemical and Pharmaceutical Bulletin*, 64(2), 83-94.
- Hayakawa, K., Tang, N., Morisaki, H., Toriba, A., Akutagawa, T., Sakai, S. (2016b) Atmospheric Polycyclic and Nitropolycyclic Aromatic Hydrocarbons in an Iron-manufacturing City. *Asian Journal of Atmospheric Environment*, 10(2), 90-98.
- Hayakawa, K., Tang, N., Toriba, A. (2017) Recent analytical methods for atmospheric polycyclic aromatic hydrocarbons and their derivatives. *Biomedical Chromatography* 31, e3862.

- Hellén, H., Kangas, L., Kousa, A., Vestenius, M., Teinilä, K., Karppinen, A., Niemi, J. V. (2017) Evaluation of the impact of wood combustion on benzo [a] pyrene (BaP) concentrations; ambient measurements and dispersion modeling in Helsinki, Finland. *Atmospheric Chemistry and Physics*, 17(5), 3475-3487.
- Huang, B., Liu, M., Bi, X. H., Chaemfa, C., Ren, Z. F., Wang, X. M., Fu, J. M. (2014) Phase distribution, sources and risk assessment of PAHs, NPAHs and OPAHs in a rural site of Pearl River Delta region, China. *Atmospheric Pollution Research*, 5, 210-218.
- Arenas-Huertero, F., Apátiga-Vega, E., Miguel-Pérez, G., Villeda-Cuevas, D., Trillo-Tinoco, J. (2011) Molecular markers associated with the biological response to aromatic hydrocarbons from urban air in humans. In *Air Pollution-New Developments*. InTech.
- International Agency for Research on Cancer (IARC) (2014) Outdoor air pollution a leading environmental cause of cancer deaths, 2013. https://www.iarc.fr/en/media-centre/iarcnews/pdf/pr221_E.pdf
- IARC Monographs of the evaluation of carcinogenic risks to humans. (2013) Diesel and Gasoline Engine Exhausts and Some Nitroarenes. Vol. 105, World Health Organization, International Agency for Research on Cancer: Lyon, France
- IARC Monographs of the evaluation of carcinogenic risks to humans. (2016) Outdoor Air Pollution. Vol. 109, World Health Organization, International Agency for Research on Cancer: Lyon, France, Retrieved from https://www.iarc.fr/en/media-centre/iarcnews/pdf/pr221_E.pdf (Accessed on November 4, 2016).
- IPCS (2003) Selected nitro- and nitro-oxy-polycyclic aromatic hydrocarbons: environmental health criteria 229. WHO, Switzerland.
http://www.who.int/ipcs/publications/ehc/229_part_I.pdf. Accessed 11 November
- International Agency for Research on Cancer (IARC) (2016) On the Evaluation of Carcinogenic Risks to Humans, Monographs, Volumes 1–116.
Retrieved from <http://monographs.iarc.fr/ENG/Classification/index.php> (Accessed on November 4, 2016)
- Jacob, P., Wilson, M., Benowitz, N. L. (2007) Determination of phenolic metabolites of polycyclic aromatic hydrocarbons in human urine as their pentafluorobenzyl ether derivatives using liquid chromatography–tandem mass spectrometry. *Analytical Chemistry*, 79, 587–598.
- Jakober, C. A., Charles, M. J., Kleeman, M. J., & Green, P. G. (2006) LC–MS analysis of carbonyl compounds and their occurrence in diesel emissions. *Analytical Chemistry*, 78, 5086–5093.
- Jariyasopit, N., Zimmermann, K., Schrlau, J., Arey, J., Atkinson, R., Yu, T. W., Dashwood, R. H., Tao, S., Simonich, S. L. M. (2014) Heterogeneous reactions of particulate matter-bound PAHs and NPAHs with NO₃/N₂O₅, OH radicals, and O₃ under simulated long-range atmospheric transport conditions: Reactivity and mutagenicity. *Environmental science & technology*, 48(17), 10155-10164.
- Kamens, R. M., Guo, J., Guo, Z., McDow, S. R. (1990) Polynuclear aromatic hydrocarbon degradation by heterogeneous reactions with N₂O₅ on atmospheric particles. *Atmospheric Environment. Part A. General Topics*, 24(5), 1161-1173.
- Kamiura, T., Kawaraya, T., Tanaka, M., Nakadoi, T. (1991) Determination of 3-nitrofluoranthene and 1-nitropyrene in suspended particulate matter by liquid chromatography with fluorescence detection. *Analytica chimica acta*, 254(1-2), 27-31.
- Kawanaka, Y., Sakamoto, k., Wang, N., Yun, S.-J. (2007) Simple and sensitive method for determination of nitrated polycyclic aromatic hydrocarbons in diesel exhaust particles by gas chromatography-negative ion chemical ionization tandem mass spectrometry. *Journal of Chromatography A*, 1163, 312-317.

- Keyte, I. J., Harrison, R. M., Lammel, G. (2013) Chemical reactivity and long -range transport potential of polycyclic aromatic hydrocarbons –a review. *Chemical Society Reviews*, 42, 9333–9391.
- Keyte, I. J., Albinet, A., Harrison, R. M. (2016) On -road traffic emissions of polycyclic aromatic hydrocarbons and their oxy - and nitro - derivative compounds measured in road tunnel environments. *Science of the Total Environment*, 566–567, 1131–1142.
- Kimata, K., Hosoya, K., Tanaka, N., Araki, T., Patterson, D. G. (1992) Preparation of nitrophenylethylsilylated silica gel and its chromatographic properties, in the separation of polychlorinated dibenzo-p-dioxins. *Journal of Chromatography A*, 595(1-2), 77-88.
- Kim, K. H., Kabir, E., Kabir, S. (2015) A review on the human health impact of airborne particulate matter. *Environment international*, 74, 136-143.
- Kojima, Y., Inazu, K., Hisamatsu, Y., Okochi, H., Baba, T., Nagoya, N. (2010) Changes in concentration levels of polycyclic aromatic compounds associated with airborne particulate matter in downtown Tokyo after introducing government diesel vehicle controls. *Asian Journal of Atmospheric Environment*, 4(1), 1-8.
- Kappos, A. D., Bruckmann, P., Eikmann, T., Englert, N., Heinrich, U., Höppe, P., Rombout, P. (2004) Health effects of particles in ambient air. *International journal of hygiene and environmental health*, 207(4), 399-407.
- Kuo, C. T., Chen, H. W., Lin, S. T. (2003) Trace determination of nitrated polycyclic aromatic hydrocarbons using liquid chromatography with on-line electrochemical reduction and fluorescence detection. *Analytica Chimica Acta*, 482(2), 219-228
- Lai, I. C., Lee, C. L., Zeng, K. Y., Huang, H. C. (2011) Seasonal variation of atmospheric polycyclic aromatic hydrocarbons along the Kaohsiung coast. *Journal of environmental management*, 92(8), 2029-2037.
- Lee, B. K., Vu VT. (2010) Sources, distribution and toxicity of polyaromatic hydrocarbons (PAHs) in particulate matter. In *Air pollution*. InTech.
- Lee, J. Y., Lane, D. A., Kim, Y. P. (2015) Formation of polyaromatic hydrocarbon (PAH) quinones during the gas phase reactions of PAHs with the OH radical in the atmosphere. *Environmental Chemistry*, 2, 307–315.
- Legzdins AE, BE McCarry and DW Bryant. (1994) Polycyclic aromatic compounds in Hamilton air: their mutagenicity, ambient concentrations and relationships with atmospheric pollutants. *Polycyclic Aromatic Compounds* 5(1-4):157-165.
- Lissowska, J., Bardin-Mikolajczak, A., Fletcher, T., Zaridze, D., Szeszenia-Dabrowska, N., Rudnai, P., Vitova, V. (2005) Lung cancer and indoor pollution from heating and cooking with solid fuels: the IARC international multicentre case-control study in Eastern/Central Europe and the United Kingdom. *American journal of epidemiology*, 162(4), 326-333.
- Li, R., Kameda, T., Li, Y., Toriba, A., Tang, N., Hayakawa, K., Lin, J. M. (2011) Hydrogen peroxide -sodium hydrosulfite chemiluminescence system combined with high -performance liquid chromatography for determination of 1-hydroxypyrene in airborne particulates. *Talanta*, 85, 2711-2714.
- Li, Z., Romanoff, L. C., Trinidad, D. A., Pittman, E. N., Hilton, D., Hubbard, K., Sjödin, A. (2014) Quantification of 21 metabolites of methylnaphthalenes and polycyclic aromatic hydrocarbons in human urine. *Analytical and Bioanalytical Chemistry*, 406, 3119–3129.
- Lin, Y., Ma, Y., Qiu, X., Li, R., Fang, Y., Wang, J., Hu, D. (2015) Sources, transformation, and health implications of PAHs and their nitrated, hydroxylated, and oxygenated derivatives in PM_{2.5} in Beijing. *Journal of Geophysical Research: Atmospheres*, 7219–7228.
- Liu, Y., Sklorz, M., Schnelle -Kreis, J., Orasche, J., Ferge, T., Kettrup, A., Zimmermann, R. (2006) Oxidant denuder sampling for analysis of polycyclic aromatic hydrocarbons and their

- oxygenated derivatives in ambient aerosol: evaluation of sampling artefact. *Chemosphere*, 62,1889-1898.
- Liu, Y. N., Tao, S., Yang, Y. F., Dou, H., Yang, Y., Coveney, R. M. (2007) Inhalation exposure of traffic police officers to polycyclic aromatic hydrocarbons (PAHs) during the winter in Beijing, China. *Science of the Total Environment*, 383, 98-105.
- Liu, Y., Chen, L., Huang, Q. H., Li, W. Y., Tang, Y. J., Zhao, J. F. (2009) Source apportionment of polycyclic aromatic hydrocarbons (PAHs) in surface sediments of the Huangpu River, Shanghai, China. *Science of the Total Environment*, 407, 2931-2938.
- Luo, K., Gao, Q., Hu, J. (2015) Derivatization method for sensitive determination of 3 hydroxybenzo[a]pyrene in human urine by liquid chromatography -electrospray tandem mass spectrometry. *Journal of Chromatography A*, 1379, 51–55.
- Lung, S. C. C., Liu, C. H. (2015) Fast analysis of 29 polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs with ultra-high performance liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry. *Scientific Reports*, 5.
- Ma, J., Chen, Z., Wu, M. H., Feng, J. L., Horii, Y., Ohura, T., Kannan, K. (2013) Airborne PM_{2.5}/PM₁₀ -associated chlorinated polycyclic aromatic hydrocarbons and their parent compounds in a suburban area in Shanghai, China. *Environmental Science and Technology*, 47, 7615-7623.
- Ma, Y., Cheng, Y., Qiu, X., Lin, Y., Cao, J., Hu, D. (2016) A quantitative assessment of source contributions to fine particulate matter (PM 2.5)-bound polycyclic aromatic hydrocarbons (PAHs) and their nitrated and hydroxylated derivatives in Hong Kong. *Environmental Pollution*, 219, 742-749.
- Mohanraj, R., Dhanakumar, S., Solaraj, G. (2012) Polycyclic aromatic hydrocarbons bound to PM 2.5 in urban Coimbatore, India with emphasis on source apportionment. *The Scientific World Journal*, 2012.
- Menichini E. 1992 Urban air pollution by polycyclic aromatic hydrocarbons: levels and sources of variability. *Science of the Total Environment*, 116(1-2): 109-35.
- Mumtaz, M. M., George, J. D., Gold, K. W., Cibulas, W., Derosa, C. T. (1996) ATSDR evaluation of health effects of chemicals. IV. Polycyclic aromatic hydrocarbons (PAHs): understanding a complex problem. *Toxicology and industrial health*, 12(6), 742-971.
- Niederer, M., (1998) Determination of polycyclic aromatic hydrocarbons and substitutes (nitro-, oxy-PAHs) in urban soil and airborne particulate by GC–MS and NCI-MS/MS. *Environ. Sci. Pollut. Res.* 5, 209–216.
- NIST (2016) Certificate of Analysis, SRM 1649b - Urban Dust, National Institute of Standards and Technology (NIST), U.S. Department of Commerce. https://www.s.nist.gov/srmors/view_detail.cfm?srm=1649B
- Nyiri, Z., Novák, M., Bodai, Z., Szabó, B. S., Eke, Z., Záray, G., Szigeti, T. (2016) Determination of particulate phase polycyclic aromatic hydrocarbons and their nitrated and oxygenated derivatives using gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 1472, 88-98.
- Ohura, T., Amagai, T., Sugiyama, T., Fusaya, M., Matsushita, H. (2004) Characteristics of particle matter and associated polycyclic aromatic hydrocarbons in indoor and outdoor air in two cities in Shizuoka, Japan. *Atmospheric Environment*, 38(14), 2045-2054.
- Ohno, T., Toriba, A., Kameda, T., Tang, N., Hayakawa, K. (2009) Determination of 1-nitropyrene in low volume ambient air samples by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A*, 1216(21), 4625-4628.
- Orakij, W., Chetianukornkul, T., Kasahara, C., Boongla, Y., Chuesaard, T., Furuuchi, M., Toriba, A. (2017) Polycyclic aromatic hydrocarbons and their nitro derivatives from indoor biomass-

- fueled cooking in two rural areas of Thailand: a case study. *Air Quality, Atmosphere & Health*, 1-15.
- Quintás, G., Lendl, B., Pastor, A., de la Guardia, M. (2008) First-order derivative resolution of overlapped PAH peaks with common mass spectra in gas chromatography–mass spectrometry. *Talanta*, 74(4), 747-752.
- Park, S. S., Kim, Y. J., & Kang, C. H. (2002) Atmospheric polycyclic aromatic hydrocarbons in Seoul, Korea. *Atmospheric Environment*, 36(17), 2917-2924.
- Perrini, G., Tomasello, M., Librando, V., Minniti, Z. (2005) Nitrated polycyclic aromatic hydrocarbons in the environment: formation, occurrences and analysis. *Annali di chimica*, 95(7), 567-578.
- Pitts, J.N., Vancauwenberghe, K.A., Grosjean, D., Schmid, J.P., Fitz, D.R., Belser, W.L., Knudson, G.B., Hynds, P.M. (1978) Atmospheric reactions of polycyclic aromatic-hydrocarbons - facile formation of mutagenic nitro-derivatives. *Science* 202, 515–519.
- Pitts, J. N., Zielinska, B., Sweetman, J. A., Atkinson, R., Winer, A. M. (1985) Reactions of adsorbed pyrene and perylene with gaseous N₂O₅ under simulated atmospheric conditions. *Atmospheric Environment*, 19(6), 911-915.
- Polichetti, G., Cocco, S., Spinali, A., Trimarco, V., Nunziata, A. (2009) Effects of particulate matter (PM₁₀, PM_{2.5} and PM₁) on the cardiovascular system. *Toxicology*, 261(1), 1-8.
- Prycek, J., Ciganek, M., Simek, Z. (2007) Clean-up of extracts for nitrated derivatives of polycyclic aromatic hydrocarbons analyses prior to their gas chromatography determination. *Journal of the Brazilian Chemical Society*, 18(6), 1125-1131.
- Ravindra, K., Bencs, L., Wauters, E., De Hoog, J., Deutsch, F., Roekens, E., Van Grieken, R. (2006) Seasonal and site-specific variation in vapour and aerosol phase PAHs over Flanders (Belgium) and their relation with anthropogenic activities. *Atmospheric Environment*, 40(4), 771-785.
- Ringuet, J. R., Albinet, A., Leoz-Garziandia, E., Budzinski, H., & Villenave, E. (2012a) Reactivity of polycyclic aromatic compounds (PAHs, NPAHs and OPAHs) adsorbed on natural aerosol particles exposed to atmospheric oxidants. *Atmospheric Environment*, 61, 15-22.
- Ringuet, J., Albinet, A., Leoz-Garziandia, E., Budzinski, H., Villenave, E. (2012b) Diurnal/nocturnal concentrations and sources of particulate-bound PAHs, OPAHs and NPAHs at traffic and suburban sites in the region of Paris (France). *Science of the Total Environment*, 437, 297-305.
- Sanderson, E. G., & Farant, J. P. (2000) Use of benzo[a]pyrene relative abundance ratios to assess exposure to polycyclic aromatic hydrocarbons in the ambient atmosphere in the vicinity of a Söderberg aluminum smelter. *Journal of the Air & Waste Management Association*, 50, 2085-2092.
- Samoli, E., Peng, R., Ramsay, T., Pipikou, M., Touloumi, G., Dominici, F., Katsouyanni, K. (2008) Acute effects of ambient particulate matter on mortality in Europe and North America: results from the APHENA study. *Environmental health perspectives*, 116(11), 1480-1486.
- Santos, A. G., Regis, A. C., Rocha, G. O., Bezerra, M. A., Jesus, R. M., Andrade, J. B. (2016) A simple, comprehensive, and miniaturized solvent extraction method for determination of particulate -phase polycyclic aromatic compounds in air. *Journal of Chromatography A*, 1435, 6-17.
- Schantz, M. M., McGaw, E., Wise, S. A. (2012) Pressurized liquid extraction of diesel and air particulate standard reference materials: effect of extraction temperature and pressure. *Analytical chemistry*, 84(19), 8222-8231.
- Snyder, L. R., Dolan, J. W., Carr, P. W. (2004) The hydrophobic-subtraction model of reversed-phase column selectivity. *Journal of Chromatography A*, 1060(1-2), 77-116.

- Šoustek, P., Michl, M., Almonasy, N., Machalický, O., Dvořák, M., Lyčka, A. (2008) The synthesis and fluorescence of N-substituted 1- and 2-aminopyrenes. *Dyes and Pigments*, 78(2), 139-147.
- Tang, N., Toriba, A., Kizu, R., Hayakawa, K. (2003) Improvement of an automatic HPLC system for nitropolycyclic aromatic hydrocarbons: Removal of an interfering peak and increase in the number of analytes. *Analytical Sciences*, 19, 249-253.
- Tang, N., Toriba, A., Kizu, R., Hayakawa, K. (2003) Improvement of an automatic HPLC system for nitropolycyclic aromatic hydrocarbons: Removal of an interfering peak and increase in the number of analytes. *Analytical Sciences*, 19, 249-253.
- Tang, N., Taga, R., Hattori, T., Tamura, K., Toriba, A., Kizu, R., Hayakawa, K. (2004) Determination of atmospheric nitrobenzanthrones by high - performance liquid chromatography with chemiluminescence detection. *Analytical Sciences*, 20, 119-123.
- Tang, N., Taga, R., Hattori, T., Toriba, A., Kizu, R., Hayakawa, K. (2005a) Simultaneous determination of twenty-one mutagenic nitropolycyclic aromatic hydrocarbons by high performance liquid chromatography with chemiluminescence detection. *Bioluminescence and Chemiluminescence*, 39(32), 441-444.
- Tang, N., Hattori, T., Taga, R., Igarashi, K., Yang, X., Tamura, K., Hayakawa, K. (2005b) Polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in urban air particulates and their relationship to emission sources in the Pan-Japan Sea countries. *Atmospheric Environment*, 39(32), 5817-5826.
- Tang, N., Araki, Y., Tamura, K., Dong, L., Zhang, X., Liu, Q., Hayakawa, K. (2009) Distribution and source of atmospheric polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in Tieling city, Liaoning Province, a typical local city in China. *Asian Journal of the Atmospheric Environment*, 3, 52-58.
- Tang, N., Araki, Y., Tamura, K., Dong, L., Zhang, X., Liu, Q., Hayakawa, K. (2009) Distribution and source of atmospheric polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in Tieling City, Liaoning Province, a typical local city in Northeast China. *Asian Journal of Atmospheric Environment*, 3(1), 52-58.
- Tang, N., Tokuda, T., Izzaki, A., Tamura, K., Ji, R., Zhang, X., Dong, L., Kameda, T., Toriba, A., Hayakawa, K. (2011) Recent changes in atmospheric polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs) in Shenyang, China. *Environmental Forensics*, 12(4), 342-348.
- Tang, N., Sato, K., Tokuda, T., Tatematsu, M., Hama, H., Suematsu, C., Kameda, T., Toriba, A., Hayakawa, K. (2014) Factors affecting atmospheric 1-, 2-nitropyrenes and 2-nitrofluoranthene in winter at Noto peninsula, a remote background site, Japan. *Chemosphere*, 107, 324-330.
- Tang, N., Hakamata, M., Sato, K., Okada, Y., Yang, X., Tatematsu, M., Hayakawa, K. (2015) Atmospheric behaviors of polycyclic aromatic hydrocarbons at a Japanese remote background site, Noto peninsula, from 2004 to 2014. *Atmospheric Environment*, 120, 144-151.
- Tang, Y. Y., Imasaka, T., Yamamoto, S., Imasaka, T. (2016) Determination of polycyclic aromatic hydrocarbons and their nitro -, amino -derivatives absorbed on particulate matter 2.5 by multiphoton ionization mass spectrometry using far-, deep-, and near-ultraviolet femtosecond lasers. *Chemosphere*, 152, 252-258.
- Tang, N., Suzuki, G., Morisaki, H., Tokuda, T., Yang, X., Zhao, L., Kameda, T., Toriba, A., Hayakawa, K. (2017) Atmospheric behaviors of particulate-bound polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons in Beijing, China from 2004 to 2010. *Atmospheric Environment*, 152, 354-361.

- Temime -Roussel, B., Monod, B., Massiani, C., Wortham, H. (2004) Evaluation of an annular denuder tubes for atmospheric PAH partitioning studies -1: Evaluation of the trapping efficiency of gaseous PAHs. *Atmospheric Environment*, 38, 1913.
- Toriba, A., Chetiyankornkul, T., Kizu, R., Hayakawa, K. (2003a) Quantification of 2 hydroxyfluorene in human urine by column-switching high-performance with fluorescence detection *Analyst*, 128, 605-610.
- Toriba, A., Kuramae, Y., Chetiyankornkul, T., Kizu, R., Makino, T., Nakazawa, H., Hayakawa, K. (2003b) Quantification of polycyclic aromatic hydrocarbons (PAHs) in human hair by HPLC with fluorescence detection: a biological monitoring method to evaluate the exposure to PAHs. *Biomedical Chromatography*, 17(2 - 3), 126-132.
- Toriba, A., Kitaoka, H., Dills, R., Mizukami, S., Tanabe, K., Takeuchi, N., Simpson, C. D. (2007a) Identification and quantification of 1 -nitropyrene metabolites in human urine as a proposed biomarker for exposure to diesel exhaust. *Chemical Research in Toxicology*, 20, 999-1007.
- Toriba, A., Hayakawa, K. (2007b) Biomarkers of exposure to polycyclic aromatic hydrocarbons and related compounds. *Journal of health science*, 53(6), 631-638.
- Toriba, A., Homma, C., Kita, M., Uozaki, W., Boongla, Y., Orakij, W., Hayakawa, K. (2016) Simultaneous determination of polycyclic aromatic hydrocarbon quinones by gas chromatography -tandem mass spectrometry, following a one -pot reductive trimethylsilyl derivatization. *Journal of Chromatography A*, 1459, 89-100.
- Turowski, M., Morimoto, T., Kimata, K., Monde, H., Ikegami, T., Hosoya, K., Tanaka, N. (2001) Selectivity of stationary phases in reversed-phase liquid chromatography based on the dispersion interactions. *Journal of Chromatography A*, 911(2), 177-190.
- Turowski, M., Yamakawa, N., Meller, J., Kimata, K., Ikegami, T., Hosoya, K., Thornton, E. R. (2003). Deuterium isotope effects on hydrophobic interactions: the importance of dispersion interactions in the hydrophobic phase. *Journal of the American Chemical Society*, 125(45), 13836-13849.
- Umbuzeiro, G.A., Franco, A., Martins, M.H., Kummrow, F., Carvalho, L., Schmeiser, H.H., Leykauf, J., Stiborova, M., Claxton, L.D. (2008) Mutagenicity and DNA adduct formation of PAH, nitro-PAH, and oxy-PAH fractions of atmospheric particulate matter from Sao Paulo, Brazil. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 652, 72-80.
- Vione, D., Barra, S., De Gennaro, G., De Rienzo, M., Gilardoni, S., Perrone, M.G., Pozzoli, L., (2004) Polycyclic aromatic hydrocarbons in the atmosphere: monitoring, sources, sinks and fate. II: sinks and fate. *Ann. Chim.* 94, 257-268.
- Wang, G., Kawamura, K., Zhao, X., Li, Q., Dai, Z., & Niu, H. (2007) Identification, abundance and seasonal variation of anthropogenic organic aerosols from a mega-city in China. *Atmospheric Environment*, 41, 407-416.
- Wang, L., Atkinson, R., Arey, J. (2007) Formation of 9,10 -phenanthrenequinone by atmospheric gas -phase reactions of phenanthrene. *Atmospheric Environment*, 41, 2025-2035.
- Wang, W., Jariyasopit, N., Schrlau, J., Jia, Y., Tao, S., Yu, T.-W., Dashwood, R.H., Zhang, W., Wang, X., Simonich, S.L.M. (2011) Concentration and photochemistry of PAHs, NPAHs, and OPAHs and toxicity of PM_{2.5} during the Beijing Olympic games. *Environ. Science & Technology*. 45, 6887-6895.
- Wang, J. Z., Ho, S. S. H., Huang, R. J., Gao, M. L., Liu, S. X., Zhao, S. Y., Han, Y. M. (2016) Characterization of parent and oxygenated-polycyclic aromatic hydrocarbons (PAHs) in Xi'an, China during heating period: An investigation of spatial distribution and transformation. *Chemosphere*, 159, 367-377.
- Wang, Q., Kobayashi, K., Wang, W. Q., Ruan, J., Nakajima, D., Yagishita, M., Terasaki, M. (2016) Size distribution and sources of 37 toxic species of particulate polycyclic aromatic

- hydrocarbons during summer and winter in Baoshan suburban area of Shanghai, China. *Science of the Total Environment*, 566–567, 1519–1534.
- Wei, C., Han, Y., Bandowe, B. A. M., Cao, J., Huang, R. J., Ni, H., Wilcke, W. (2015) Occurrence, gas/particle partitioning and carcinogenic risk of polycyclic aromatic hydrocarbons and their oxygen and nitrogen containing derivatives in Xi'an, central China. *Science of the Total Environment*, 505, 814–822.
- WHO (2006), WHO Air Quality Guidelines: Global Update 2005, WHO/SDE/PHE /OEH/06.02
- Wingfors, H., Hägglund, L., Magnusson, R. (2011) Characterization of the size -distribution of aerosols and particle -bound content of oxygenated PAHs, PAHs, and n -alkanes in urban environments in Afghanistan. *Atmospheric Environment*, 45, 4360–4369.
- Watanabe, T., Hirayama, T. (2001) Genotoxicity of soil. *Journal of Health Science*, 47(5), 433–438.
- Wei, C., Han, Y.M., Bandowe, B.A.M., Cao, J.J., Huang, R.-J., Ni, H., Tian, J., Wilcke, W., (2015a) Occurrence, gas/particle partitioning and carcinogenic risk of polycyclic aromatic hydrocarbons and their oxygen and nitrogen containing derivatives in Xi'an, central China. *Science of the Total Environment*. 505, 814–822.
- Wei, C., Bandowe, B.A.M., Han, Y., Cao, J., Zhan, C., Wilcke, W., (2015b) Polycyclic aromatic hydrocarbons (PAHs) and their derivatives (alkyl-PAHs, oxygenated-PAHs, nitrated PAHs and azaarenes) in urban road dusts from Xi'an, Central China. *Chemosphere* 134, 512–520.
- Wu, S., Yang, B., Wang, X., Hong, H., Yuan, C. (2012) Diurnal variation of nitrated polycyclic aromatic hydrocarbons in PM₁₀ at a roadside site in Xiamen, China. *Journal of Environmental Sciences*, 24(10), 1767–1776.
- Yaffe, D., Cohen, Y., Arey, J., Grosovsky, A.J., (2001) Multimedia analysis of PAHs and nitroPAH daughter products in the Los Angeles Basin. *Risk Analysis*. 21, 275–294.
- Yaffe, D., Cohen, Y., Arey, J., Grosovsky, A.J., (2001) Multimedia analysis of PAHs and nitroPAH daughter products in the Los Angeles Basin. *Risk Analysis*, 21, 275–294.
- Yang, H. H., Chien, S. M., Chao, M. R., Wang, Y. F. (2005) Size distribution of particulate polycyclic aromatic hydrocarbons in the diluted four-stroke motorcycle exhausts. *Aerosol and Air Quality Research*, 5(2), 225–235.
- Zhang, D., Zhu, D., Chen, W., (2009) Sorption of nitroaromatics to soils: comparison of the importance of soil organic matter versus clay. *Environment Toxicology Chemistry*, 28, 1447–1454
- Zhang, M., Xie, J. F., Wang, Z. T., Zhao, L. J., Zhang, H., Li, M. (2016) Determination and source identification of priority polycyclic aromatic hydrocarbons in PM_{2.5} in Taiyuan, China. *Atmospheric Research*, 401, 178–179.
- Zhang, W., Zhuang, G. S., Huang, K., Li, J., Zhang, R., Wang, Q. Z., Wang, W. (2010) Mixing and transformation of Asian dust with pollution in the two dust storms over the northern China in 2006. *Atmospheric Environment*, 44, 3394.
- Zielinska, B., McDonald, J. D., Whitney, K., Lawson, D. R. (2004) Emission rates and comparative chemical composition from selected in use diesel and gasoline fueled vehicles. *Journal of the Air and Waste Management Association*, 54, 1138–115.
- Zielinska, B., Samy, S. (2006) Analysis of nitrated polycyclic aromatic hydrocarbons. *Analytical and bioanalytical chemistry*, 386(4), 883–890.
- Zimmermann, K., Jariyasopit, N., Simonich, S.L.M., Tao, S., Atkinson, R., Arey, J., (2013) Formation of nitro-PAHs from the heterogeneous reaction of ambient particle-bound PAHs with N₂O₅/NO₃/NO₂. *Environmental Science and Technology*, 47, 8434–8442.

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